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SEPTEMBER 1953
VOLUME 56 NUMBER 3

Published Monthly by
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

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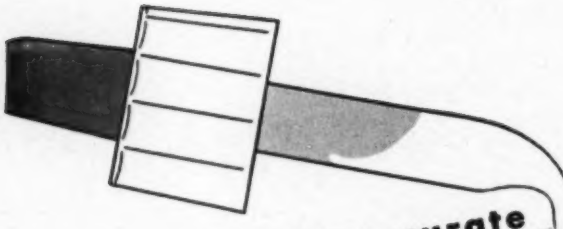

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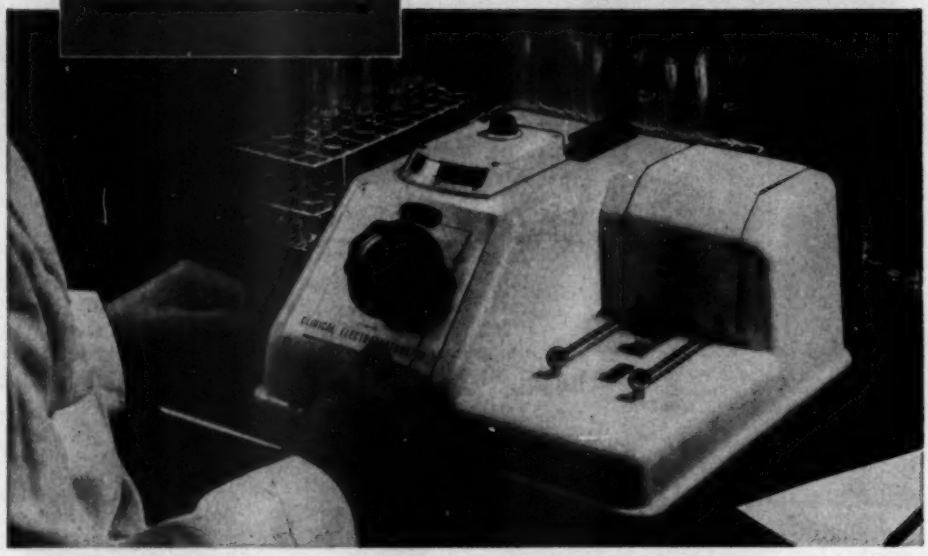

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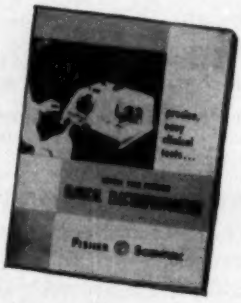

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PROTECTIVE INFLUENCE OF PREGNANCY ON EXPERIMENTAL COLLAGEN DISEASE LESIONS

RUSSELL L. HOLMAN, M.D.
AND
CHESTER K. JONES, M.D.
NEW ORLEANS

THE FACT that the crippling effects of rheumatoid arthritis are greatly lessened by pregnancy was first recognized over 100 years ago. This ameliorative influence of pregnancy prompted the classical studies of Hench, Kendall, Slocumb, and Polley[1] on the relationship of the adrenal gland to rheumatoid arthritis. Since then cortisone and related compounds have been tried in all types of collagen disease with and without pregnancy. While it is generally recognized by clinicians that collagen diseases may improve during pregnancy, the clinical literature on this subject is surprisingly meager, and no publications on the effects of pregnancy on experimental collagen disease lesions in animals could be found. This report records a definite protective influence of pregnancy on experimental necrotizing arteritis in dogs in relation to diet and renal insufficiency.

METHODS

Arterial lesions that closely resemble those of rheumatic arteritis and periarteritis nodosa in man have been produced with regularity in dogs by feeding a standard high-fat diet for eight weeks or longer, then inducing standard renal insufficiency.[2] The standard diet—calf liver (raw wet weight) 32 parts, sugar 25 parts, starch 25 parts, and commercial creamery butter 18 parts—is essentially a high-fat, low-protein diet with 43% of its caloric value derived from fat, 7% from protein, and 50% from carbohydrate. One gram of salt mixture[3] and 5 gm. of kaolin were added to each daily diet. The vast majority of mongrel dogs readily consumed this diet and showed an average gain in body weight of about 10% when it was fed for eight weeks in amounts to furnish 75 calories per kilogram per day.

Bilateral nephrectomy and heavy metal injury (1.0 ml. per kilogram of 0.8% aqueous solution of uranyl nitrate injected subcutaneously, usually in the anterior chest wall) have proved equally effective in precipitating typical arterial lesions in properly fed dogs. The dogs stopped

This work was aided by grants from the Life Insurance Medical Research Fund and from the National Heart Institute.

From the Department of Pathology, Louisiana State University School of Medicine, and Charity Hospital of Louisiana.

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1. Hench, P. S.: Ann. Int. Med. **36**:1, 1952. Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Proc. Staff Meet., Mayo Clin. **24**:181, 1949

2. Holman, R. L.: Am. J. Path. **17**:359, 1949; ibid. **19**:147, 1943; ibid. **19**:159, 1943; ibid. **19**:977, 1943. Holman, R. L., and Swanton, M. C.: Proc. Soc. Exper. Biol. Med. **63**:87, 1946. Holman, R. L.: Ibid. **66**:307, 1947.

3. McCollum, E. V., and Simmonds, N. A.: J. Biol. Chem. **33**:55, 1918.

eating on the 2nd to the 5th day and died in "uremia" on the 4th to the 14th day following the induction of standard renal insufficiency. No consistent changes in blood pressure have been observed at any time during the experimental procedure.[4]

The accidental observation that one dog was pregnant and failed to show typical arterial lesions suggested additional experiments to determine the influence of pregnancy. Since the 63-day gestation period of dogs closely parallels the time necessary for a satisfactory experiment, the first problem was to obtain dogs as soon after conception as possible. Attempts to mate dogs under kennel conditions proved successful in only one instance (Dog 51-26). The other four

TABLE 1.—*Incidence of Arterial Lesions in Nonpregnant Dogs Fed Standard Diet and Subjected to Standard Renal Insufficiency*

Dog No.	Sex	Standard Diet Before SRI,* Weeks	Death, Days After Induction of SRI	Terminal Blood Urea Nitrogen, Mg./100 Cc.	Typical Arterial Lesions †
49-41	M	8	11	181	+++
49-45	F	8	10	288	+++
49-67	F	8	6	118	+++
49-68	M	8	13	200	+++
49-69	F	8	12	255	+++
49-71	F	8	10	264	+++
49-72	M	8	6	264	+++
49-76	M	8	14	192	+
50-26	F	8	8	360	+++
50-63	F	8	9	312	++
50-130	F	8	6	140	+++
50-112	M	8	5 ‡	348	+++
50-114	M	10	5 ‡	220	+++
50-116	M	8	4 ‡	240	+

* SRI, standard renal insufficiency produced by subcutaneous injection of 8.0 mg./kg. of uranyl nitrate in 0.8% aqueous solution.

† Key to symbols: +++, largest gross lesion, 1 cm. or more in maximum dimension; ++, largest lesion seen on gross examination, less than 1 cm. in maximum dimension; +, lesion discovered in routine histological sections.

‡ Standard renal insufficiency produced by bilateral nephrectomy.

TABLE 2.—*Incidence of Arterial Lesions in Pregnant Dogs Fed Standard Diet and Subjected to Standard Renal Insufficiency*

Dog No.	Standard Diet Before SRI,* Weeks	Days After Induction of SRI		Terminal Blood Urea Nitrogen, Mg./100 Cc.	Typical Arterial Lesions
		Delivery of Pups	Death of Mother		
50-127	7 †	8	7	232	0
50-152	8	2	12	152	0
51-26	8	3	6	207	0
51-33	8	0	6	206	0
51-42	8	2	7	95	0

* SRI, standard renal insufficiency produced by subcutaneous injection of uranyl nitrate, 8.0 mg./kg. of uranyl nitrate in 0.8% aqueous solution.

† During first 8 weeks the standard diet was fed. During the last two weeks the amount of butter in the standard diet was doubled.

successful pregnancies in this series were salvaged from nine dogs that were reported by the dog delivery man to have been in heat and "exposed to the neighborhood" shortly before being brought to the kennel.

In order to insure adequate intake of the dietary toxicomponent, the amount of butter was doubled during the seventh week of the experiment for three of the dogs and during the last two weeks for another dog (Table 2). Other experiments in nonpregnant dogs have shown that

4. Holman, R. L., in Factors Regulating Blood Pressure, Transactions of the Fourth Conference, February 23-24, 1950, edited by B. W. Zweifach and E. Shorr, New York, Josiah Macy, Jr., Foundation, 1951, p. 150.

doubling the amount of butter in the standard diet did not halve the period of dietary feeding but did reduce it from eight weeks to five or six weeks.[5] Whenever the amount of butter was doubled, the diet was kept isocaloric by reducing the amounts of sugar and starch.

All dogs in both pregnant and nonpregnant groups were killed when they became moribund and necropsies were held shortly after death. Careful gross and microscopic examinations

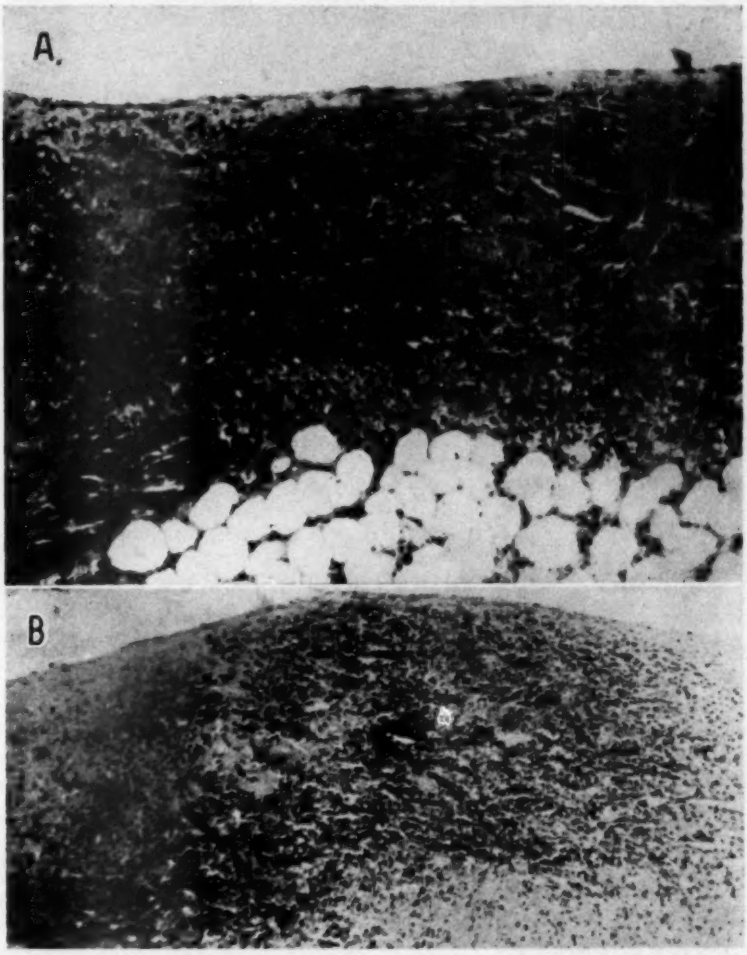

Fig. 1.—*A* (Dog 49-67), acute necrotizing left auriculitis. Hematoxylin and eosin; × 150. *B* (Dog 49-71), acute necrotizing arteritis of main pulmonary artery. Hematoxylin and eosin; × 100.

with special attention to the cardiovascular system were carried out in all instances. For additional details of methods the reader is referred to previous publications.[6]

5. Holman, R. L.: Unpublished data.

6. (*a*) Holman, R. L.: South, M. J. **42**:108, 1949; (*b*) in Karsner, H. T., and Sanford, A. H., Editors: 1951 Year Book of Pathology and Clinical Pathology, Chicago, Year Book Publishers, Inc., 1952, p. 101. (*c*) Footnote 2.

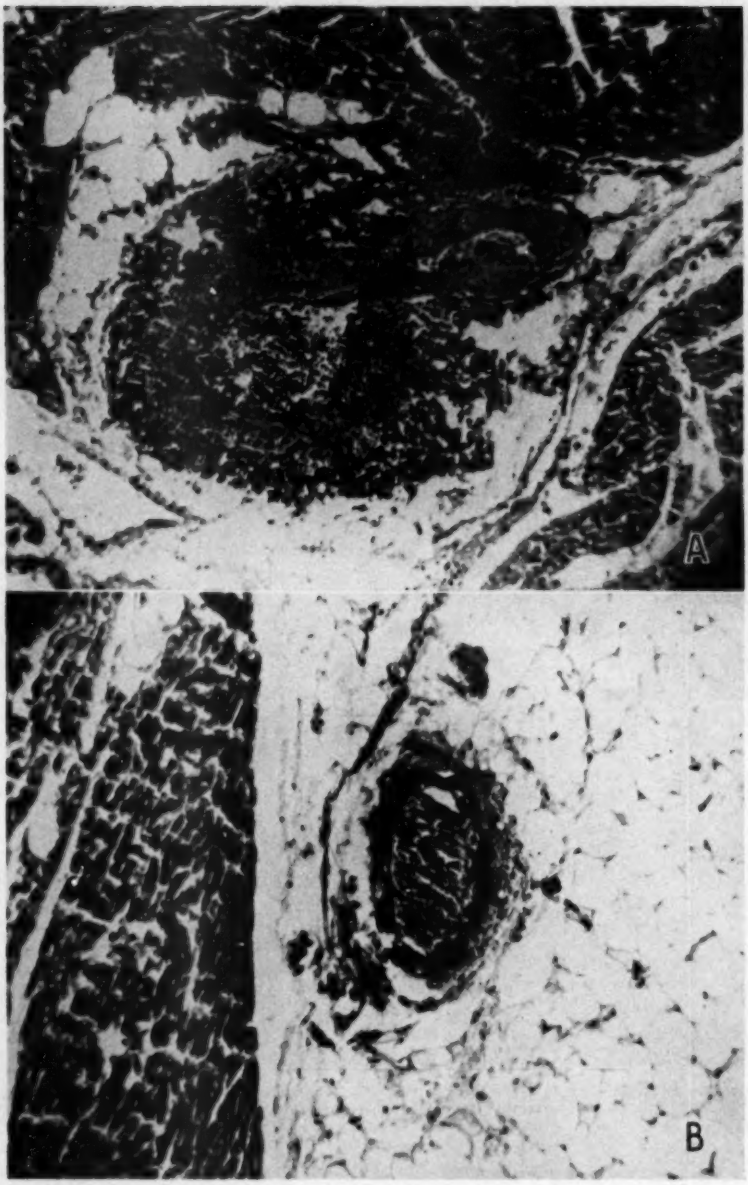

Fig. 2.—*A* (Dog 49-71), acute necrotizing arteritis of coronary artery. Hematoxylin and eosin; × 120. *B* (Dog 49-67), acute necrotizing arteritis of coronary arteriole in subepicardial fat. Hematoxylin and eosin; × 120.

EXPERIMENTAL OBSERVATIONS

Prevention of lesions leaves nothing spectacular to show for the effort, yet prevention remains one of the highest ideals of medicine. In order to appreciate the results of these experiments it is first necessary to show examples of what happened in the control, or nonpregnant, group. Typical lesions in the endocardium of the left auricle, in an elastic artery, in a muscular artery, and in an arteriole are illustrated in Figures 1 and 2. These typical arterial lesions exhibit all the features of necrotizing arteritis that characterize the collagen diseases in man. None of these lesions were found in the pregnant dogs.

The results of these experiments are unusually clear-cut and are summarized in Tables 1, 2, and 3. Whereas all 14 nonpregnant dogs (including 7 females of the same size and age group as the pregnant dogs) developed typical arterial lesions, none of the 5 pregnant dogs that were subjected to the same experimental procedure developed arterial lesions.

COMMENT

Two important questions in the discussion of these experimental findings arise: (*a*) What is the mechanism of the protection afforded to the arterial system of

TABLE 3.—*Protective Influence of Pregnancy on Arterial Lesions in Dogs Fed Standard High-Fat Diet and Subjected to Standard Renal Insufficiency*

Group	No. of Dogs in Group	No. with Arterial Lesions	% Positive for Arterial Lesions
Nonpregnant	14	14	100
Pregnant	5	0	0

dogs by the pregnant state? (*b*) Do these experimental findings in dogs have any human counterpart? While neither question can be answered definitely, there are some pertinent data on each.

Possible Mechanisms by Which the Pregnant State Protects Dogs Against Experimental Collagen Disease Lesions.—The basic mechanisms involved in the experimental procedures (standard high-fat diet plus standard renal insufficiency causes typical arterial lesions) are not known, and space does not permit a discussion of the various mechanisms that have been postulated.[6] It suffices to state that all the evidence points to too much of something in the diet and too little of something from the kidneys. Most of the available data are consistent with the following hypothesis: Primary injury to the arterial wall is produced by the peroxidation of certain unsaturated fatty acids that pile up in the tissues during the eight weeks of standard high-fat diet. Normally this peroxidation of fatty acid is prevented by fat-soluble antioxidants acting synergistically with one or more co-factors (citric acid? phosphoric acid? adenosine?) secreted by the proximal convoluted tubules of the kidney.

Since the lesions have never been observed in the absence of kidney damage, the following question naturally arises: Could the fetal kidneys contribute a factor of safety similar to that afforded by the fetal pancreas in maternal diabetes? With this thought in mind, the blood and kidneys of several of the puppies were examined at intervals up to five days after delivery (five to seven days after injecting uranyl

nitrate into the subcutaneum of the mother). The blood showed a progressive azotemia (up to 70 mg. per 100 cc.), and the newborn kidneys showed evidence of coagulative necrosis of the proximal convoluted tubules. All the evidence indicated that the fetal kidneys were also damaged and were not in a condition to furnish the factor of safety postulated.

Other studies (unpublished, but briefly referred to [6b]) have shown that cortisone, corticotropin, and diethylstilbestrol in appropriate dosage have prevented the development of typical arterial lesions that regularly follow the induction of standard renal insufficiency in properly fed dogs. It seems more logical, therefore, to attribute the protective influence of pregnancy demonstrated in the above experiments to enhanced activity of the maternal endocrines possibly augmented by some activity of the fetal endocrine system (none of the glands of internal secretion appeared to be damaged by uranyl nitrate). This explanation admittedly dodges the issue, for the mechanism of hormonal protection is not known, but the available evidence points to a hormonal factor rather than to protection afforded by fetal kidneys.

Experimental Findings in Dogs and Human Counterpart.—Attempts to discover from the literature the effect of pregnancy on the course of collagen diseases other than rheumatoid arthritis met with surprisingly little success. Concerning lupus erythematosus, Eastman [7] states that in many instances pregnancy exerts a beneficial though transitory effect on the lesions. The occurrence of scarlet fever and acute glomerulonephritis with pregnancy is known to be rare.[8] Scleroderma in the one case we found reported was perhaps temporarily arrested during pregnancy.[9] Acute rheumatic fever, especially first attacks, is known to be a rare complication of pregnancy.[10] The paucity of papers on this subject may be an indication that the collagen diseases rarely arise during pregnancy.

An attempt was made to review the records of all cases of collagen diseases in women in the age group 12 to 50 years, inclusive, during the decade 1941-1951 in the Charity Hospital of Louisiana. The results of this attempt were disappointing to say the least and are illustrated in the first collagen disease reviewed. Of the first 100 consecutive cases filed under the heading of rheumatoid arthritis, 26 had "acceptable" roentgenographic and other evidence for the diagnosis. Seven of these 26 patients had no recorded history of pregnancy, and 4 other patients gave a history of onset of arthritic symptoms after menopause or after delivery of the last baby. Of the remaining 15 "acceptable" records, 13 contained no mention of the effects of pregnancy on the arthritic manifestations, leaving only two cases in which definite ameliorative effects of pregnancy were recorded. The results were much the same when all cases filed under rheumatoid arthritis were "accepted," and similar unsatisfactory results were obtained with the next collagen disease studied, rheumatic fever. The review was not completed, for it was realized that such studies in order to be significant must be planned in advance with particular attention to such points as first attack of collagen disease, month of gestation, association factors, therapy, et cetera. It is hoped that the experimental results recorded in this paper will stimu-

7. Eastman, N. J.: Williams Obstetrics, Ed. 10, New York, Appleton-Century-Crofts, Inc., 1950, p. 751.

8. Diddle, A. W.; Trussel, R. E., and Plass, E. D.: Am. J. Obst. & Gynec. **39**:608, 1940.

9. Guttmacher, A. F.: Urol. & Cutan. Rev. **47**:107, 1943.

10. Hollander, A. G., and Goldsmith, J. W., Jr.: Am. J. Obst. & Gynec. **42**:333, 1941.

late such forward-looking clinical research and that answers to such questions as to whether or not current indications for interrupting pregnancy take cognizance of a protective influence of pregnancy in the collagen diseases will be forthcoming.

To summarize the question under discussion, the experimental findings in dogs having any human counterpart, it is fair to state that the meager data that we have found in the literature and the still more meager data obtained from the files of the Charity Hospital of Louisiana afford some confirmation for a protective influence of pregnancy in human cases of collagen disease but leave much room for future study.

SUMMARY

Arterial lesions that closely resemble those of rheumatic arteritis, polyarteritis nodosa, and other collagen diseases in man occurred in 14 of 14 nonpregnant dogs that were fed a standard high-fat diet for eight weeks or longer and then subjected to standard renal insufficiency.

None of five pregnant dogs subjected to the same experimental procedure developed arterial lesions.

The mechanism of this protective influence of pregnancy has not been determined, but available evidence points to a hormonal factor rather than to protection afforded by fetal kidneys.

Relative data pertaining to human subjects have been briefly reviewed.

AGING PROCESSES OF AORTA AND PULMONARY ARTERY IN NEGRO AND WHITE RACES

Comparative Study of Various Segments

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DURING the past century three important concepts have been formulated regarding the pathogenesis of arteriosclerosis. The first was presented by Virchow,[1] who believed the process to be partially inflammatory, and his theory in modified form still has some adherents. The second, championed by Thoma and others,[2] held that the medial coat undergoes primary degenerative changes followed by a compensatory proliferation of the intima. The cause of these degenerative processes was considered to be the outcome of excessive functional demand or the consequence of the wear and tear of age. The third, dealing with faulty lipid metabolism as the primary etiological factor, originated with Aschoff,[3] who showed that the doubly refractile bodies in atheroma were similar to cholesterol esters and postulated that they infiltrated from the blood stream. He was able to simulate this process by feeding rabbits a high-cholesterol diet.

The concepts of Virchow and Thoma continue to hold the attention of some investigators, despite the great emphasis at the present time on the influence of lipid-metabolic factors in the genesis of atherosclerotic plaques. With regard to the inflammatory concept, the syphilitic lesion of the aorta, essentially adventitial and medial, is commonly associated with large intimal plaques; the anatomical distribution of the lesions is, however, somewhat different from that in arterio-

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†Dr. Gray died Aug. 18, 1949.

These investigations were supported by grants from the David May-Florence G. May Research Foundation and the Louis Monheimer Fund.

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1. Virchow, R.: Aus dem pathologisch-anatomischer Curse des Prof. Rudolf Virchow: VII. Der atheromatose Prozess der Arterien, Wein. med. Wchnschr. **6**:809-812, and 825-827, 1856.

2. Thoma, R., and Kaefer, N.: Über die Elasticität gesunder und kranker Arterien, Virchows. Arch. path. Anat. **116**:1-27, 1889. Faber, A.: Die Arteriosklerose, Jena, Gustav Fischer, 1912. Beitzke, H.: Zur Entstehung der Atherosklerose, Virchows Arch. path. Anat. **267**:625-647, 1928.

3. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924.

sclerosis. Furthermore, Paterson, Slinger, and Gartley [4] have observed an inflammatory reaction in arteriosclerosis in the chicken, and Yater and co-workers [5] believe that coronary arterial disease in relatively young persons may have an inflammatory component. Three independent lines of investigation have been reported with regard to the role of degenerative phenomena of the media. Two of these studies deal with the medial lesions as a factor in determining the locus of formation of atherosclerotic plaques. Baldwin, Taylor, and Hass [6] have studied the effects of physical factors which injure the elastic elements; on the other hand, the investigations of Waters and de Suto-Nagy [7] have dealt with injury to the arterial wall produced by epinephrine (Adrenalin) and other substances as the localizing factors, while at the same time they utilized various agents which alter lipid transport in the blood stream to develop superimposed atheromatous plaques.

All of the foregoing, whether dealing with medial changes as possible localizing factors or with circulating lipid-metabolic factors, which may produce atheromatous plaques, fail to explain adequately the peculiar distribution of lesions in the "spontaneous" human disease. As we have previously pointed out,[8] they do not account for the predilection of this disease for certain areas of the aorta and the cerebral and coronary arteries, as well as for the arterial tree of the lower extremities, while sparing primarily the pulmonary arterial system and, to a less extent, the hepatic, splenic, and renal arteries. In order to understand better the nature of this distribution, we have been carrying out a series of investigations dealing with differences in the pattern of distribution of the elastic tissue and the intensity of its degeneration, regeneration, and calcification as a function of age (wear and tear) in various arterial systems.[9]

Initially, in our investigations the results appeared to indicate, as in certain of the foregoing reports, that medial calcification was an effect of wear and tear on elastic elements which predisposed to the formation of atheromatous plaques. Subsequent studies, however, indicated that this process of elastic tissue calcification

4. Paterson, J. C.; Slinger, S. J., and Gartley, K. M.: Experimental Coronary Sclerosis: Medical Degeneration as the Primary Lesion in Coronary Sclerosis of Cockerels, Arch. Path. **45**:306-318, 1948.

5. Yater, W. M.; Traum, A. H.; Brown, W. G.; Fitzgerald, R. P.; Geisler, M. A., and Wilcox, B. B.: Coronary Artery Disease in Men 18 to 39 Years of Age: Report of 866 Cases, 450 with Necropsy Examinations, Am. Heart J. **36**:683-722, 1948.

6. Baldwin, D.; Taylor, C. B., and Hass, G. M.: A Comparison of Arteriosclerotic Lesions Produced in Young and in Old Rabbits by Freezing the Aorta, Arch. Path. **50**:122-131, 1950.

7. Waters, L. L., and de Suto-Nagy, G. I.: Lesions of the Coronary Arteries and Great Vessels of the Dog Following the Injection of Adrenalin: Their Prevention by Dibenamine, Science **111**:634-635, 1950.

8. Handler, F. P.; Blache, J. O., and Blumenthal, H. T.: Comparison of Aging Processes in the Renal and Splenic Arteries in the Negro and White Races, A. M. A. Arch. Path. **53**:29-53, 1952.

9. (*a*) Blumenthal, H. T.; Lansing, A. I., and Wheeler, P. A.: Calcification of the Media of the Human Aorta and Its Relation to Intimal Arteriosclerosis, Aging and Disease, Am. J. Path., **20**:665-687, 1944. (*b*) Lansing, A. I.; Blumenthal, H. T., and Gray, S. H.: Aging and Calcification of the Human Coronary Artery, J. Gerontol. **3**:87-97, 1948; (*c*) The Interrelation of Elastic Tissue and Calcium in the Genesis of Arteriosclerosis, Am. J. Path. **26**:989-1009, 1950. (*d*) Blache, J. O., and Handler, F. P.: Coronary Artery Disease: A Comparison of the Rates and Patterns of Development of Coronary Arteriosclerosis in the Negro and White Races and Its Relation to Clinical Coronary Artery Disease, Arch. Path. **50**:189-198, 1950. (*e*) Footnote 8.

also participated intimately in the formation of the intimal lesions and that the sites of this participation appeared to determine the focus of formation of intimal atheromata. In some instances the intensity of these changes varied with racial groups; differences in the incidence of formation of atheromatous plaques in the Negro and white races in the instance of the coronary arteries corresponded to a markedly diminished incidence of myocardial infarction in Negroes, while in the renal arteries the increased incidence of hypertension in the latter group was associated with more severe calcification of elastic elements.

Since the aorta and pulmonary artery show such striking differences in the frequency of atheromatosis, they were chosen as the material for the present study. Because Rosenthal[10] has reported differences in average measurements of the aorta at various levels between Negro and white races, it was thought advisable to continue the comparative racial study in these vessels along lines previously reported.[9] The present report further deals with differences in the aging (wear and tear) changes in the elastic tissue and its mineralization in the pulmonary artery and in various segments of the aorta as they may relate to differences in physical forces which prevail in these various locations.

TABLE 1.—*Age Distribution of Patients*

Age Group, Yr.	Pulmonary Artery		Ascending Aorta		Thoracic Aorta		Abdominal Aorta	
	White	Negro	White	Negro	White	Negro	White	Negro
0 to 20	11	1	2	3	5	2	5	1
21 to 40	15	11	1	2	9	14	10	12
41 to 60	46	21	13	12	16	22	16	21
61 to 80	64	14	11	10	26	19	26	15
81 and over	12	1	3	3	4	1	4	1
Total	148	48	30	30	60	58	61	50

MATERIAL AND METHOD

The numerical distribution of cases in each race, according to vessel and regional segment of the aorta, is shown in Table 1. No separation is made according to sex, since no significant difference in intensity of elastic tissue calcification was observed in the two sexes. Horizontal blocks were taken from the pulmonary artery and the ascending aorta about 2 cm. distant to the respective valves. Segments of thoracic aorta were taken about midway between the origin of the left subclavian artery and the diaphragm and from the abdominal aorta, just below the level of the origin of the renal arteries.

Specimens were fixed in formaldehyde solution U. S. P. diluted 1:10 in absolute alcohol. Sections were prepared by the usual paraffin-embedding technique and cut at a thickness of 5 to 7 μ. One section was stained with hematoxylin and eosin; an adjacent section was stained with the Weigert-Verhoeff stain for elastic tissue, and a third section was incinerated for a study of mineral distribution by dark-field illumination. For each specimen a description of the elastic tissue pattern was made, as well as a record of the pattern and intensity of mineral distribution; the latter was graded 1 to 4 +, as previously described.[9a,b]

In addition, a series of pulmonary and aortic segments were obtained from white persons only, at the same levels noted above. The intima and adventitia were stripped from the media, and the percentage of calcium of the medial coat was determined according to the method

10. Rosenthal, S. R.: Studies in Atherosclerosis: Chemical, Experimental and Morphological: III and IV. Roles of Cholesterol Metabolism, Blood Pressure and Structure of the Aorta: the Fat Angle of the Aorta (F. A. A.), and the Infiltration-Expression Theory of Lipid Deposit, Arch. Path. **18**:660-698, 1934.

utilized by Lansing, Alex, and Rosenthal [11] for calcium content of elastin. These determinations served as a quantitative check for the more subjective method utilized in grading incinerated sections. The age distribution of these patients is shown in Table 2.

RESULTS

Description of Aging Changes.—Age Group 0 to 20 Years: In all of the various segments of the aorta, except an abdominal segment in one instance, there was no evidence of subendothelial thickening. The endothelium was a unicellular layer overlying an intact internal elastic lamella. In the one exception there was a fibroblastic and collagenic intimal layer occupying about one-fifth of the thickness of the wall. In all instances the elastic fibers of the media were closely packed and thick and showed graceful undulations. Small fibrils branched from these fibers, but no granular material taking the elastic stain was noted. Microincinerated sections revealed a mineral pattern corresponding to the nuclei of the medial muscle, with a thin band of calcium corresponding in position to the internal elastic lamella (Fig. 1*A*, *B*, *E*, and *F*). No differences were noted between the three segments of the aorta, and there was no difference observed between the two races.

TABLE 2.—*Age Distribution of Patients for Quantitative Determination of Medial Calcium*

Age Group, Yr.	Pulmonary Artery	Ascending Aorta	Thoracic Aorta	Abdominal Aorta
0 to 20	5	3	5	5
21 to 40	8	6	8	8
41 to 60	19	15	20	20
61 to 80	20	37	22	22
81 and over	1	4	1	1
Total	53	65	56	56

During the first decade of life the internal elastic lamella of the pulmonary artery was in a position similar to that observed in the aorta. In the media there was a uniform distribution of undulating elastic fibers. No external elastic lamella was indentified in any age group. During the second decade the position of the internal lamella remained unchanged, but the elastic fibers of the media, which were arranged almost as compactly as in the aorta during the first decade, became more widely spaced, and thin fibrils were seen branching from them. Microincinerated specimens generally showed a nuclear mineral pattern of the medial nuclei and a thin line of calcium corresponding to the internal lamella. However, in one case of pulmonary tuberculosis there was a 1+ calcification sharply distinguishing the elastic fibers. Again, no differences were noted between the two races.

Age Group 21 to 40 Years: The intimal layer was essentially the same in the ascending and thoracic segments of the aorta. The endothelium consisted of a single layer of cells, A layer of collagen (20 to 40% of the thickness of the wall) appeared between the endothelium and the internal elastic lamella. The latter was well defined, but elastic fibrils extended into the collagen layer (Fig. 1*D*). In contrast, in the abdominal segment the fibrils extending into the intima were coarser and more numerous, many of these coursing in a direction parallel to the parent membrane; in many instances the outlines of the latter became indistinct.

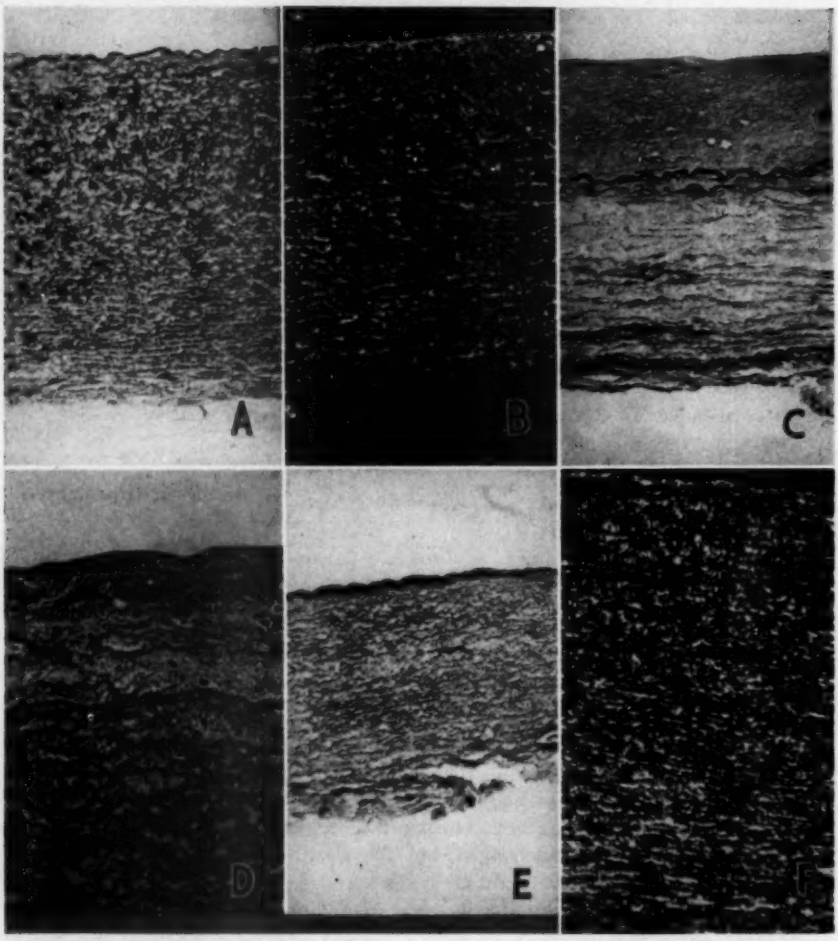

Fig. 1.—*A* and *B*, thoracic aorta, 15-year-old white boy. The internal elastic lamella lies against the endothelium and shows early calcification (accentuation of elastic line). Medial fibers appear intact and mineral pattern of media is nuclear; × 100. *C*, thoracic aorta, 35-year-old white man. There is extensive hyaline thickening of the intima, which is almost devoid of elastic elements. Despite some duplication, the internal elastic lamella is easily identified. Media exhibits an "atrophic" pattern; × 100. *D*, thoracic aorta, 50-year-old white man. Intima here also shows extensive thickening but is rich in elastic structures. Despite the extensive increase in elastic elements in both intima and media, there is a fairly sharp separation of these layers by a definable internal elastic lamella. This is in contrast to Figure 2*C* and *D*, which illustrates an abdominal segment; × 100. *E* and *F*, abdominal aorta, 15-year-old white girl. Elastic tissue preparation (*E*); × 100. Incinerated specimen (*F*); × 150. The elastic tissue pattern is similar to that of Figure 1 *A* and *B*, although there is more fraying of medial elastic elements, and the mineral pattern of the media is slightly heavier than the nuclear pattern.

Definite fenestrations in the membrane developed, and granules of elastic-staining material were noted in many of these spaces. The subendothelial layer thus formed also occupied about 20 to 40% of the wall (Fig. 2*A* and *B*). Occasional plaques containing cholesterol slits and lipid-staining macrophages were encountered, and in such areas elastic fibrils were found between the plaque and the endothelium, as well as at the base of the focus of lipid deposition.

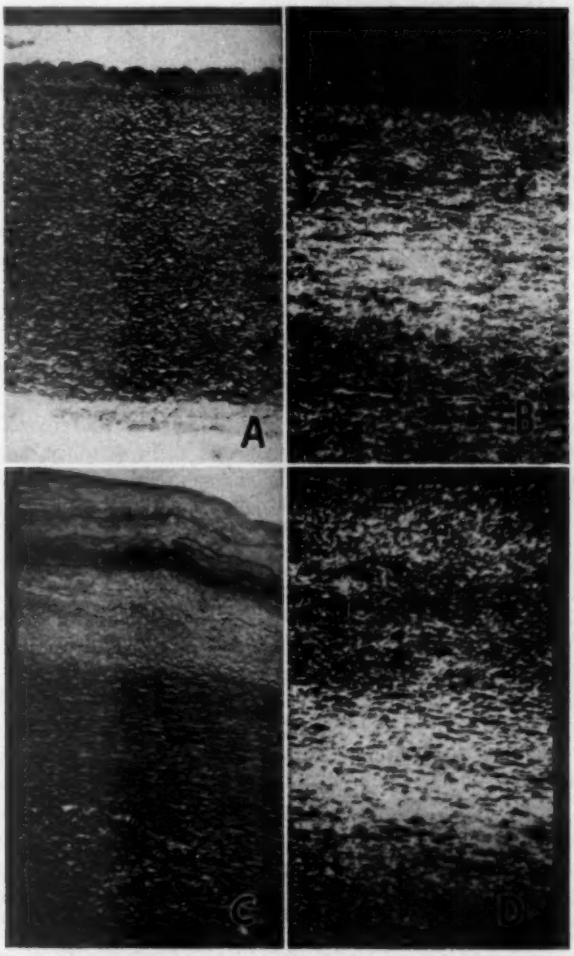

Fig. 2.—*A* and *B*, abdominal aorta, 39-year-old Negro man. Elastic tissue preparation (*A*); × 100. Incinerated specimen (*B*); × 150. There is mild thickening of the intima, which contains numerous elastic elements. The internal elastic lamella can still be made out but is not as sharply defined as in the thoracic aorta. There is considerable fraying of elastic fibers of the media and almost 3 + mineralization. *C* and *D*, abdominal aorta, 60-year-old white man. These sections exhibit a "zoning" phenomenon. There are alternating layers of collagen with few elastic filaments and bands of densely packed elastic structures. Correspondingly, there are alternating layers of light and heavy mineralization; × 100.

In the media of all aortic segments, with the exception of the abdominal segments noted below, coarse fragments and small fibrils were found apparently branching from elastic fibers. Deposits of granular material taking the elastic stain were noted between elastic fibers and fibrils. This change occurred first in the inner third of the media but gradually involved the entire medial coat (Fig. 2*A* and *B*). In the ascending and descending thoracic segments of the aorta calcification of these elements averaged 1 +, but in the abdominal segment it averaged 2 +. In occasional segments, both thoracic and abdominal, there was a separation of elastic fibers with intervening collagen and muscle and relatively few elastic fragments and granules (Fig. 1*C*). Again, the inner half of the media was first involved, with subsequent deeper spread, and calcification of the elastic elements averaged 2 +. However, the strata of collagen and muscle, with few elastic elements, showed less than 1 + calcification.

In the pulmonary artery there was no development of a subendothelial layer of collagen except in two cases: one of mitral stenosis with severe chronic passive congestion of the lungs and the second a case of organizing pneumonitis. Elastic fibers continued to be plump, with little filament and no granule formation, and, in general, the wider spatial arrangement of elastic fibers was maintained. Except for the two cases noted above, there was a barely perceptible increase in calcium along the inner margin of elastic tissue and in the deeper media. In the two cases of pulmonary disease there were foci of elastic filament and granule formation, and these areas showed 2 + calcification.

As in the previous age group, there was no distinct difference between the two racial groups. However, in the Negro group there was one case of diabetes in which there were severe medial elastic changes and 4 + calcification in the abdominal segment and a case of syphilitic aortitis with a nuclear mineral pattern of the aortic media.

Age Group 41 to 60 Years: In the ascending and descending thoracic segments the subendothelial layer of collagen and elastic filaments generally occupied 10 to 20% of the thickness of the wall. The internal lamella consisted of a band, varying in thickness, of tightly packed slender filaments and granules extending into the subendothelial layer. Plaques were only rarely encountered. Calcification along these lamellae averaged 1 +. In contrast, in the abdominal segment the internal elastic lamella was frayed, fragmented, and usually broken beyond identification as a unit; granules and fine fibrils were distributed in a fairly discrete zone, replacing the lamella. The subendothelial collagenic layer varied between 20 and 40% of the thickness of the wall (Fig. 2*C* and *D*). Plaques containing cholesterol slits and calcium deposits were frequently observed, and small filaments and granules of elastic-staining material were found overlying, beneath, and within the plaques.

Two patterns of arrangement of elastic tissue were again observed in the media in both the thoracic and the abdominal segments. One consisted of an increase in coarse fragments, filaments, and granules, with a loss of sharp outlining of the elastic fibers, and the other consisted of thin, delicate, straightened fibers widely separated from each other. The intervening areas of the latter were composed of muscle and collagen with a few elastic filaments or granules. Calcification in the first type was generally 1 + higher than in the second type. The former variety was termed the "elastoid" type and the latter, the "atrophic" pattern. Some vessels

showed a mixed reaction, with an elastoid pattern in some areas and an atrophic change in others. Calcification of the medial elastin of the thoracic segments averaged 1 + in white persons and 1.5 + in Negroes, while in the abdominal segments the average was slightly over 2 + in the white race and 3 + among Negroes.

In the pulmonary artery the internal lamella in all patients consisted of several compactly arranged parallel undulating bands of elastic fibers. Subendothelial collagenic thickening of significant degree was encountered in only six patients, and in all of these there was associated pulmonary disease, either in the form of long-standing inflammation or of hypertension due to valvular heart disease; even in the latter it never exceeded 20% of the thickness of the wall, and only rarely were extensions of filaments and granules noted in the intimal layer. The spacing of the medial elastic fibers was variable. In those pulmonary arteries in which spacing was wider the fibers were generally thinner than in those with a more compact pattern. Intervening filaments and granules were occasionally observed in focal collections, and these showed 1 + calcification; generally, however, calcification was barely more intense than that which we have called a nuclear mineral pattern (Fig. 3*C* and *D*).

Age Group 61 to 80 Years: The internal lamella of both thoracic segments remained essentially unchanged from the previous age group. The subendothelial layer averaged 15% of the thickness of the wall, and calcification along the internal lamella averaged slightly more than 1 + in the white race. These changes were more intense in Negroes, in whom the average calcification exceeded 2 +. On the other hand, the thickness of the subendothelial layer in the abdominal aorta averaged about 15% of the thickness of the wall; the internal lamella was seldom identifiable as a single band but consisted rather of a poorly outlined zone of delicate elastic filaments, fragments, and granules varying in thickness in different areas; where plaques were encountered, this elastic material was found overlying them and spreading from the base of such elevations into the media. This accounted for zones of calcification at the base and overlying plaques. It is of special interest that calcium deposits did not surround cholesterol slits (Fig. 3*A* and *B*). Calcification along the internal lamellar zone averaged somewhat over 2 + in both races.

The two patterns of change in medial elastic tissue described in the previous age group were found also in the segments studied in this age group. The "elastoid" pattern was by far the most frequent, and these vessels showed an average 3 + calcification in both races. The average calcification in segments showing the "atrophic" pattern was only 2 +, and there was no significant racial difference. Secondary areas of atrophy of elastic elements were seen in several vessels, giving rise to a zonal pattern with alternating bands of high and low mineral content. The penetration of plaques into the media also produced foci of elastic atrophy.

Age Group 81 Years and Older: Thin subendothelial bands of collagen, never exceeding 10% of the thickness of the wall, were present in most pulmonary arteries in this age group, and these bands were generally free of elastic elements, except when pulmonary disease was associated with alteration of the pulmonary arteries. However, in all instances the internal lamella remained well defined; in nondiseased lungs the internal lamella was hardly mineralized, while in some diseased lungs calcification averaged 1 +. Most striking were the medial changes. Medial elastic

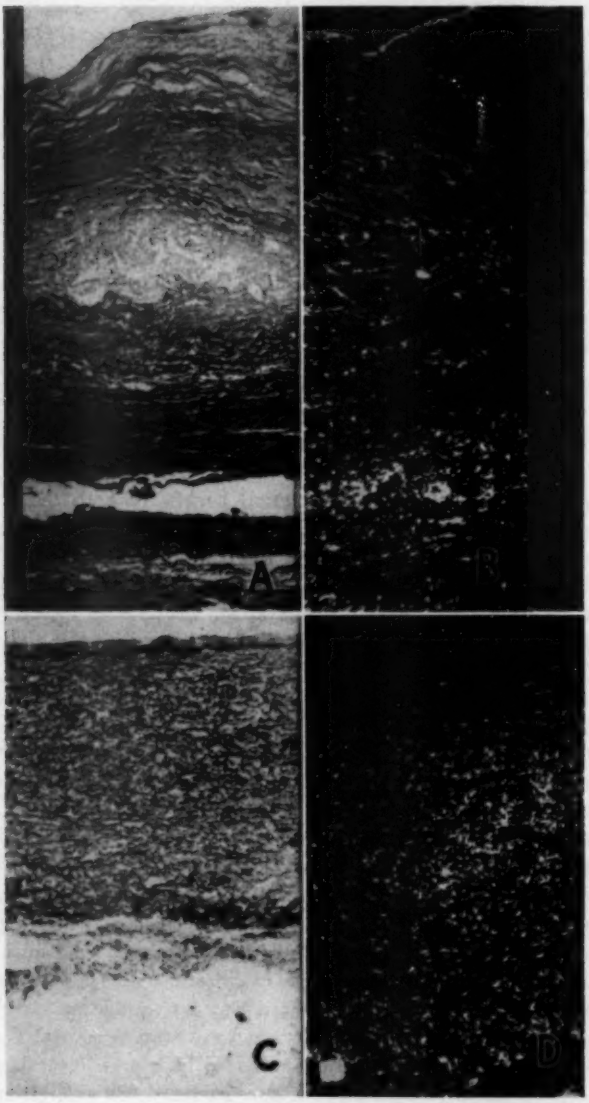

Fig. 3.—*A* and *B*, abdominal aorta, 63-year-old white man. Section shows a large area of lipid deposition surrounded on all sides by collagen containing elastic fibers and filaments. The medial elastic structures are compressed. Mineral deposition is present above and below, but little mineral is noted within the area of lipid deposition; × 100. *C* and *D*, pulmonary artery, 57-year-old white man. There is a relatively thin intimal layer and a sharply defined internal elastic lamella. Medial elastic structures are intact, for the most part, and mineral deposition is slightly less than 1 +; × 100.

fibers remained essentially the same as in the previous age group in arteries from normal lungs and showed less than 1% mineralization, while in arteries from diseased lungs there was fairly marked formation of filaments and granules, along with thinning of the parent fibers, and these frequently showed better than 2 + calcification (Fig. 4*C* and *D*).

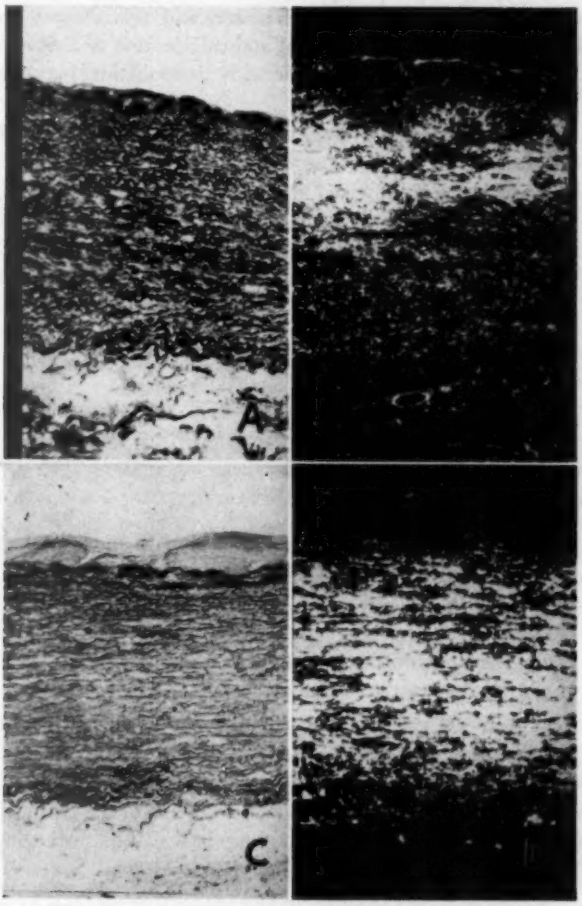

Fig. 4.—*A* and *B*, pulmonary artery, 87-year-old white man. Fragmentation and filamentation of medial elastic fibers are much more marked than in Figure 3*C* and *D*, but many intact fibers are still present. Mineral deposition of media is 3 + in some areas. Despite advanced age of the patient, the internal elastic lamella is distinct and the intima is thin; × 100. *C* and *D*, pulmonary artery, 70-year-old white man with mitral stenosis. There is an increased thickness of the intimal layer and marked fragmentation of elastic elements of the media. Note also marked thinning of the internal elastic lamella. The media shows a diffuse 3 + mineral deposition; × 100.

The subendothelial layer of collagen was found to occupy, on the average, only 10 to 15% of the thickness of the wall in the thoracic segments but 30 to 40% in the abdominal segment. Plaques were encountered in the thoracic segments, but these rarely comprised more than 40% of the thickness of the wall, while in the

abdominal segment they frequently occupied as much as 70% of the wall. The elastic tissue changes along the internal lamellar zone were not qualitatively different from the previous age group. Calcification along this zone ranged from 1 to 2+ in the thoracic segments and averaged 3+ in the abdominal segment; there was no significant racial difference. The commonest medial elastic pattern was a mixture of the two types previously described; uniform atrophy of the medial elastic elements was found in only one instance, and this showed the narrowest subendothelial collagen layer and alteration and calcification of the internal lamellar zone. In the mixed types calcification occurred in three distinct bands, as previously described (Fig. 2*C* and *D*).

In the pulmonary artery the average subendothelial layer occupied one-fifth of the thickness of the wall. There was no distinct change over the previous 20-year period, and in several cases the internal lamella remained as a single plump, slightly wavy band just beneath the lining endothelium. About one-third of the cases showed an "atrophic" medial elastic pattern, and the remaining two-thirds showed moderate deposition of filaments and granules, with about a 2+ calcification (Fig. 4*A* and *B*). Again, there was no significant racial difference.

TABLE 3.—*Comparison of Ash and Calcium Content of the Media of the Pulmonary Artery and Various Segments of the Aorta*

	Pulmonary Artery		Ascending Aorta		Descending Aorta		Abdominal Aorta	
Age Group, Yr.	Average % Ash*	Average % Ca*	Average % Ash*	Average %Ca*	Average % Ash*	Average % Ca*	Average % Ash*	Average % Ca*
0 to 20.....	1.38	0.13	1.23	0.14	1.22	0.14	1.80	0.13
21 to 40.....	2.49	0.19	2.86	0.57	3.76	0.78	4.34	0.81
41 to 60.....	2.74	0.35	5.39	1.17	5.66	1.22	5.54	1.32
61 to 80.....	3.55	0.42	8.37	2.25	7.39	1.94	6.41	1.73
81 and over.....	3.60	0.46	10.87	3.89	8.98	2.58	7.64	2.59

* Calculated on the basis of dry weight of media.

COMPARISON OF PERCENTAGE OF ASH AND CALCIUM IN DRIED MEDIA OF THE PULMONARY ARTERY AND VARIOUS SEGMENTS OF THE AORTA

It is apparent from the results tabulated in Table 3 that both the percentage of ash and the percentage of calcium in the media of all vessels studied progressively increased with age. The segments of the pulmonary artery trunk contained proportionately less ash and calcium than the various segments of the aorta in corresponding age groups, except for the youngest age group in which the percentage of calcium was remarkably constant in all arteries. These data, therefore, corresponded to those obtained by microscopic study. Similar correspondence between microscopic observations and quantitative chemical determinations were also obtained in the various segments of the aorta, up to the age of 40 years; thus, in general, the lowest percentage of ash was found in the ascending aorta, with the descending thoracic segments showing slightly higher values, while the greatest concentrations of ash and calcium were obtained from segments of the abdominal aorta.

However, such a correlation failed after the age of 40 years. While the concentrations of ash and calcium were lowest in the ascending aorta in the age group 41 to 60 years, there was no significant difference between the data obtained on the descending thoracic and abdominal segments. The lack of correspondence between

microscopic estimation and quantitative determination of ash and calcium was even more striking in the last two age groups; in these, the greatest concentrations of ash and calcium were found in the segments of ascending aorta, which, of the aortic segments, would be expected to contain the least mineral, and the lowest in the abdominal segments, which would be expected to contain the largest quantities of ash. There was no significant difference between the percentage of calcium in the descending thoracic and abdominal aorta in the oldest age group, but the percentage of ash was higher in the descending thoracic segment. The reasons for these apparent discrepancies will be presented in the discussion.

COMMENT

On the basis of these histological studies it appears that two similar processes occur in the pulmonary trunk and the various segments of the aorta but that they progress at different rates. One occurs in the region of the internal elastic lamella, and the second occurs in the region of the elastic fibers of the media. In both, the changes consist of a splitting, thinning, and fraying of elastic fibers, forming fibrils, small fragments, and eventually a fine granular material which continues to exhibit an affinity for elastic stains. Along with these alterations there is a progressive increase in mineral, primarily calcium, either on the surface or within the elastic elements.

Not only do these changes take place at different rates in the two zones noted above but also they occur at different rates in different vessels and even in various segments of the same artery. Thus it is apparent from both the histological and the chemical phases of the present study that the changes in the media of the pulmonary artery take place much more slowly than in the aorta. In the latter vessel it would appear from histological observations that medial calcification occurs most rapidly in the abdominal segment, less rapidly in the descending thoracic portion, and least rapidly in the ascending aorta. The lack of correspondence of the chemical data is due in part to a relative increase in collagen, which results in a lowered proportion of ash and calcium but not in an actual diminution in the quantity of these elements. The experiments of Lansing, Alex, and Rosenthal [11] with elastin freed of the other structural elements of the media have shown a progressive increase in the percentage of calcium associated with elastic tissue with increasing age. Further, the greatest relative increase in collagen occurred in those vessels showing an "atrophic" pattern, and such vessels played an appreciable role in lowering the average per cent of ash and calcium. An additional source of error in older vessels lay in the difficulty in obtaining a sharp cleavage between intima and media. In vessels with advanced intimal changes and loss of identity of the internal elastic lamella, such a separation was extremely difficult, and undoubtedly portions of media were lost with the discarded intima. Similar objections to stripping procedures in analyzing medial processes have been raised by Buck and Rossiter [12] and by Prior and Jones.[13]

Furthermore, genetic factors in the sense of racial differences may condition the severity of these changes. In previous reports differences between Negro and white

11. Lansing, A. I.; Alex, M., and Rosenthal, T. B.: Calcium and Elastin in Human Arteriosclerosis, J. Gerontol. **2**:112-119, 1950.

12. Buck, R. C., and Rossiter, R. J.: Lipids of Normal and Atherosclerotic Aortas: A Chemical Study, A. M. A. Arch. Path. **51**:224-237, 1951.

13. Prior, J. T., and Jones, D. B.: Structural Alterations Within the Aortic Intima in Infancy and Childhood, Am. J. Path. **28**:937-952, 1952.

groups in the severity of aging processes were demonstrated in the coronary and renal arteries.[14] In the present investigations distinct differences were noted only in the aorta, and these were most marked in the abdominal segment. Such differences were most easily discernible in the age group 41 to 60; in subsequent age groups elastic tissue calcification was of such severity in most specimens that racial differences, if present, were obscured. In general, these observations correspond to those of Rosenthal,[10] who reported that the abdominal segment of the aorta of Negroes shows a 60 to 69% increase in width between that of age groups 25 to 71, as compared with a 39 to 40% increase in a white group. Also, in Rosenthal's studies the incidence of arteriosclerosis of the aorta was, in general, higher in the Negro group than in the White group.

In our early reports,[9a,b] on this subject it was concluded that medial calcification predisposed to the formation of intimal plaques since it preceded the latter and since atheromata were not observed without some degree of medial calcification. Subsequent observations have led to some modification of this view. The various ways in which medial calcification may affect the formation of intimal plaques have been discussed in previous reports[14]; even in those vessels which do not show a predominance of elastic elements in the medial coat at an early age, there may develop a considerable quantity of elastic material in the media with senescence. While these changes may play a role in the formation of intimal plaques, it now appears that the changes along the internal elastic lamella are more directly connected with the actual formation of such plaques. Thus, in the pulmonary artery not only do the medial elastic changes occur at a slow rate but also the internal elastic lamella remains intact even in aged subjects, and elastic fibrils extending into the intima are only rarely encountered. The pulmonary artery nevertheless is capable of developing both severe medial and intimal elastic-calcium changes in persons who, for one reason or another, have pulmonary hypertension. There is also a striking difference in the changes along the internal elastic lamella in the various segments of the aorta. The internal elastic lamella remains identifiable well into the senescent period in the ascending aorta; while it disappears more rapidly in the thoracic segment, it remains identifiable for a much longer period in the latter location than in the abdominal aorta. The loss of identity results from marked splitting, fraying, and fragmentation, with extension of these elastic elements into the intima. These changes seem to occur concomitantly with the laying down of collagen in the abdominal aorta and constitute the earliest formation of a true intimal layer. In the higher segments of the aorta this expansion of elastic elements lags behind the deposition of collagen, but we have not observed the presence of lipid-containing macrophages in the absence of such elastic tissue changes.

An evaluation of the role of lipid-metabolic factors in the genesis of human arteriosclerosis must take into account the sequence of appearance of the various components which constitute the intimal plaque. The validity of comparing experimentally produced intimal lesions with the human atheromatous plaque must likewise depend upon the order of appearance of elastic elements, collagen, changes in ground substance, and lipids. Jores[15] recognized the fact that in youth and early adult life the endothelium of the aorta rests on a thick and prominent internal elastic

14. Footnotes 8 and 9*d*.

15. Jores, L.: Arteriosklerose, in Henke, F., and Lubarsch, O., editors: Handbuch der speziellen pathologischen: Anatomie und Histologie, Berlin, Springer-Verlag, 1924, p. 703.

lamella and that in later life a layer of fibrous tissue intervenes between the endothelium and the internal elastic lamella, either uniformly over wide areas or in patches. Recently Prior and Jones [13] have observed similar intimal thickenings in a group of young persons at autopsy. Torhorst,[16] as well as Ehlers [17] and Giroux,[18] observed similar changes in the pulmonary arteries and with Jores agreed that atheromatous changes usually begin in such areas of intimal thickening. Rhinehart and Greenberg's [19] observations on the sequence of appearance of the various components of the intimal plaque agree with our observations, namely, that the early changes consist of cellular proliferation and deposition of collagen and elastic tissue to which they add the accumulation of mucoid substance. Lipids appear later and are found usually in the deeper layers of the thickened intima, and their presence is often found in close association with degenerative changes in cells, intercellular substances, and possibly elastic elements.

On the basis of such observations we have suggested that lipids may be released within the wall of arteries as a by-product of these degenerative changes, rather than by infiltration of lipids carried in the blood stream.[8] This would account for a lower incidence of atheromatous plaques in vessels showing a lesser degree of these intimal alterations. That the infiltration of lipids from the blood stream does not constitute a likely mechanism is indicated by the fact that natural or experimental factors which elevate the circulating lipid level indiscriminately produce atheromata of the pulmonary, as well as the systemic, arteries, unlike the naturally occurring human disease. Furthermore, Roger, Binet, and Verne [20] showed that after intravenous injection of olive oil in dogs the fat globules are arrested in the small pulmonary arteries. These investigators stated that this process occurs normally in the case of postprandial hyperlipemia, but, since after meals the amount of fat in the systemic veins is increased, it seems that much of it is so finely divided that it passes through the pulmonary capillaries. Similarly, MacMahon and Weiss [21] showed that in carbon tetrachloride poisoning the fat released from the liver goes to the pulmonary circulation, forming a layer which constitutes 64% of the total pulmonary blood volume. If these observations apply to humans, then hyperlipemia does not appear to be a major factor in human arteriosclerosis, since the pulmonary circulation should be the most susceptible area, when, in fact, it is the most resistant.

Pulmonary arteriosclerosis has, of course, long been recognized. It appears to be primarily associated with high pulmonary arterial pressure, although infections, intoxications, and congenital defects may play a role. In Posselt's [22] series, 40%

16. Torhorst, H. R.: Die histologischen Veränderungen bei Sklerose der Pulmonalarterien, Beitr. path. Anat. **36**:210-241, 1904.

17 Ehlers, H. W. E.: Zur Histologie der Arteriosklerose der Pulmonalarterie, Virchows Arch. path. Anat. **178**:427-446, 1904.

18. Giroux, L.: Sclérose et athérome de l'artère pulmonaire: Rôle des conditions mécaniques, Arch. mal. coeur **3**:218-235, 1910.

19. Rinehart, J. F., and Greenberg, L. D.: Arteriosclerotic Lesions in Pyridoxine-Deficient Monkeys, Am. J. Path. **25**:481-491, 1949; Pathogenesis of Experimental Arteriosclerosis in Pyridoxine Deficiency: With Notes on Similarities to Human Arteriosclerosis, A. M. A. Arch. Path. **51**:12-18, 1951.

20. Roger, H.; Binet, L., and Verne, J.: Le processus histologique de la lipodiérèse pulmonaire, Compt. rend. Soc. biol. **88**:1140-1141, 1923.

21. MacMahon, H. E., and Weiss, S.: Carbon Tetrachloride Poisoning with Macroscopic Fat in the Pulmonary Artery, Am. J. Path. **5**:623-630, 1929.

22. Posselt, A.: Die klinische Diagnose der Pulmonalarteriensklerose, München med. Wchnschr. **55**:1625-1629, 1908.

of cases of pulmonary arteriosclerosis were associated with mitral stenosis. Wartman [23] found severe pulmonary sclerosis in 16.9% of men and 17.5% of women over the age of 60. On the other hand, Brenner [24] believes that some degree of pulmonary arteriosclerosis is present in all patients over 40. Nevertheless, it is generally agreed that, while pulmonary vascular sclerosis increases in frequency and severity with age, only in exceptional instances is it as severe as in the systemic circulation, and its frequency is considerably lower than in the latter.

While the medial changes described in the present study may influence the intimal changes only indirectly, they nevertheless may represent a more massive response to physical forces applied to the vessel wall. Burton [25] has recently quantitated such forces for vessels of various sizes by applying the Laplace principle. In his studies, blood vessels are considered as cylinders open at both ends and filled with a fluid that has a greater hydrostatic pressure than that existing outside the vessel walls. The hydrostatic pressure within the vessel acts at right angles to the wall, tending to distend the vessel and increase its diameter. This force is opposed by the tension in the wall of the vessel, tangential at every point, tending to diminish the diameter of the vessel. The total tension may consist of several components: (1) elastic tension due to stretch of the vessel wall and the elastic fibers, (2) active tension due to contraction of the smooth muscle elements in the wall, and (3) interfacial tension due to possible "surface tension" between the fluid in the vessel and wall. The total tension, "T," is the product of the radius and the hydrostatic pressure within. It appears likely, therefore, that the difference in medial and also in intimal changes between the pulmonary artery and aorta may be accounted for in the difference in "T" that is effective over a period of years. As Brenner [24] points out, the systolic pressure in the pulmonary artery is about one-sixth of that in the aorta. This tension thus becomes a principal aging (wear and tear) factor. We have recently [26] applied the Laplace law to the various segments of the arterial tree of the lower extremity, and there appears to be a high degree of correlation between the effective tension and the frequency of atheromatous plaques, as well as severity of medial changes at the various levels in the arterial tree of the lower extremity.

This, then, may constitute an example of how physical forces may affect histological structures and chemical processes in tissues. In the present study we have dealt primarily with the effect of such forces on the elastic elements. The prevailing tension level may in large part determine the elastic tissue response. Occasionally a high tension may be associated with thick, prominent elastic fibers from which finer filaments may disappear completely, giving rise to an "atrophic" pattern. The latter may also occur from the replacement of elastic tissue by fibrous tissue, particularly from pressure of a large intimal plaque. In general, however, in arteries operating under relatively high tensions there appears to be an increase in total elastic tissue with age, although much of it may be in a fibrillar or granular form. On the other hand, a low operating tension may also result in an atrophy of elastic

23. Wartman, W. B.: The Incidence and Severity of Arteriosclerosis in the Organs from 500 Autopsies, Am. J. M. Sc. **186**:27-35, 1933.

24. Brenner, O.: Pathology of the Vessels of the Pulmonary Circulation, Arch. Int. Med. **56**:1189-1241, 1935.

25. Burton, A. C.: On the Physical Equilibrium of Small Blood Vessels, Am. J. Physiol. **164**:319-329, 1951.

26. Pareira, M. D.; Handler, F. P., and Blumenthal, H. T.: Aging Processes in the Arterial and Venous Systems of the Lower Extremities, Circulation **8**:36-43, 1953.

elements, as in the case of the pulmonary artery, in which with increasing age the elastic fibers become thinner. While there is some fibrillar change in pulmonary arteries, there appears to be a loss of total elastic tissue with age. It appears, therefore, that high operating tensions may actually serve as a stimulus to the formation of elastic tissue.

However, the origin of the increase in elastic elements in systemic arteries is largely unknown. Recent studies by Rinehart and Greenberg [19] may bear on this subject. These investigators point out that mucopolysaccharides are found not only in the reticular tissues of the dermis, the synovial tissues, tendon sheaths, and heart valves but also in blood vessels where they ensheath elastic tissue fibers and lie between the smooth muscle cells of the media. These investigators have observed that in the Macacus rhesus monkey, pyridoxine deficiency leads to a swelling of mucoid material in arteries, with the development of lesions similar to those in human arteriosclerosis. According to K. Meyer,[27] the mucopolysaccharides of heart valves, tendon, and aorta (structures rich in elastic fibers) differ from those of other tissues containing mesenchymal elements, since the former contain chondroitin sulfates B and C. This may indicate a relation between these types of mucopolysaccharide and the formation of elastic elements. It has been apparent in our studies that considerably more elastic tissue appears in systemic arteries as the process of arteriosclerosis becomes severer than can be accounted for on the basis of splitting off of elastic elements from parent elastic membranes. The new formation of elastic fibers and fibrils about a new lumen formed in the process of recanalization of a thrombus appears to occur in a mucoid bed. Such observations suggest a new area of investigation concerning the origin of elastic tissue, in both the embryo and the adult, a study which may be expected to yield new information relating to the genesis of arteriosclerosis.

SUMMARY

This report is a continuation of studies dealing with the effects of aging (wear and tear) on the elastic structures of arteries. A comparison is presented of the pattern of distribution of elastic tissue in the pulmonary artery and various segments of the aorta, as well as the intensity of degeneration, regeneration, and calcification of elastic elements at various age periods. Histochemical techniques, as well as quantitative determinations, of the percentage of ash and calcium of the media have been utilized.

The elastic tissue-calcium changes are least severe in the pulmonary artery, except in cases of pulmonary hypertension, and are progressively severer in the ascending aorta, descending thoracic aorta, and finally the abdominal aorta, in that order. The average intensity of these processes is greater in the Negro group than in the white group between ages 41 and 60; in younger and older groups no significant racial differences have been noted.

The significance of these observations is discussed in its relation to the genesis and distribution of intimal atheroma. Particular emphasis is placed on the role of physical forces applied to the vessel wall, as calculated by the formula of Laplace, and to the effects of such forces operating over relatively long periods of time. Possibilities as to the origin of newly formed elastic elements are also discussed.

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27. Meyer, K.: Chemistry of Connective Tissue Polysaccharides, in Connective Tissues, Transactions of the First Conference, April 24-25, 1950, New York, Josiah Macy, Jr. Foundation, 1950, pp. 89-100.

SUBACUTE CEREBRAL FAT EMBOLISM COMPLICATED BY JUXTAEMBOLIC THROMBOSIS OF FIBRIN

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THE FORMATION of fibrin thrombi adjacent to fat emboli was noted in the glomeruli of the kidney by Waaler,[1] but the explanation was equivocal. The unique relationship was recently observed in the kidneys and in the brain in another case of fatal fat embolism, which is reported in this study. This opportunity permitted a discussion of the mechanism involved in the production of such thromboemboli, as well as of perivenous fibrin depositions.

REPORT OF CASES

CASE 1 (0.35/50).—*Clinical History.*—A 47-year-old farmer had fractured his right leg in a traffic accident. He gave a complete account of the incident four and one-half hours later when admitted to the hospital. He complained of chills. Cyanosis and a condition of shock were noted; the pulse rate was 60 with small amplitude, and the blood pressure was 70/50. The pupils were equal and reacted sluggishly to light and in accommodation. The patient underwent an operation in which the fragments of the fractured tibia and fibula were replaced and maintained in position by the application of a bone plate, using Lane's technique. Complete immobilization was obtained by a plaster cast. A blood transfusion and dextrose injections were administered. After an uneventful postoperative course, the patient became comatose on the second hospital day, i.e., 31 hours after the accident. The plantar response became dorsiflexed on the left foot. The pupils of the eyes became pinhole in size and showed no reaction to light. Additional dextrose injections were given. On the second day petechial hemorrhages occurred on the body. On the third hospital day, the patient remained in a coma; the blood urea was 150 mg. per 100 cc.; the hemoglobin was 86%; the erythrocyte count was 4,160,000; the hematocrit reading was 31%. The patient died 77 hours after the accident, following continuous coma of 46 hours.

Postmortem Examination.—The lungs, kidney, and brain had multiple petechial hemorrhages. Sudan-stained frozen sections of these organs demonstrated numerous fat emboli in capillaries and in larger sinusoidal vessels as in the glomeruli of the kidney and in the area postrema of the brain (Fig. 1). The multiple emboli of the cerebral grey and white matter had here and there resulted in the development of small round areas of necrosis. In the cerebral cortex and in the tegmentum of the midbrain, isolated small round foci of acute nerve cell disappearance were noted. The loss of nerve cells resulted in the occurrence of pale foci and around such foci histiocytic cells had been activated to form microglial cells. In the nuclei

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1. Waaler, E.: Histological Changes in the Kidneys in Fat-Embolism, Acta path. et microbiol. scandinav. **20**:329-334, 1943.

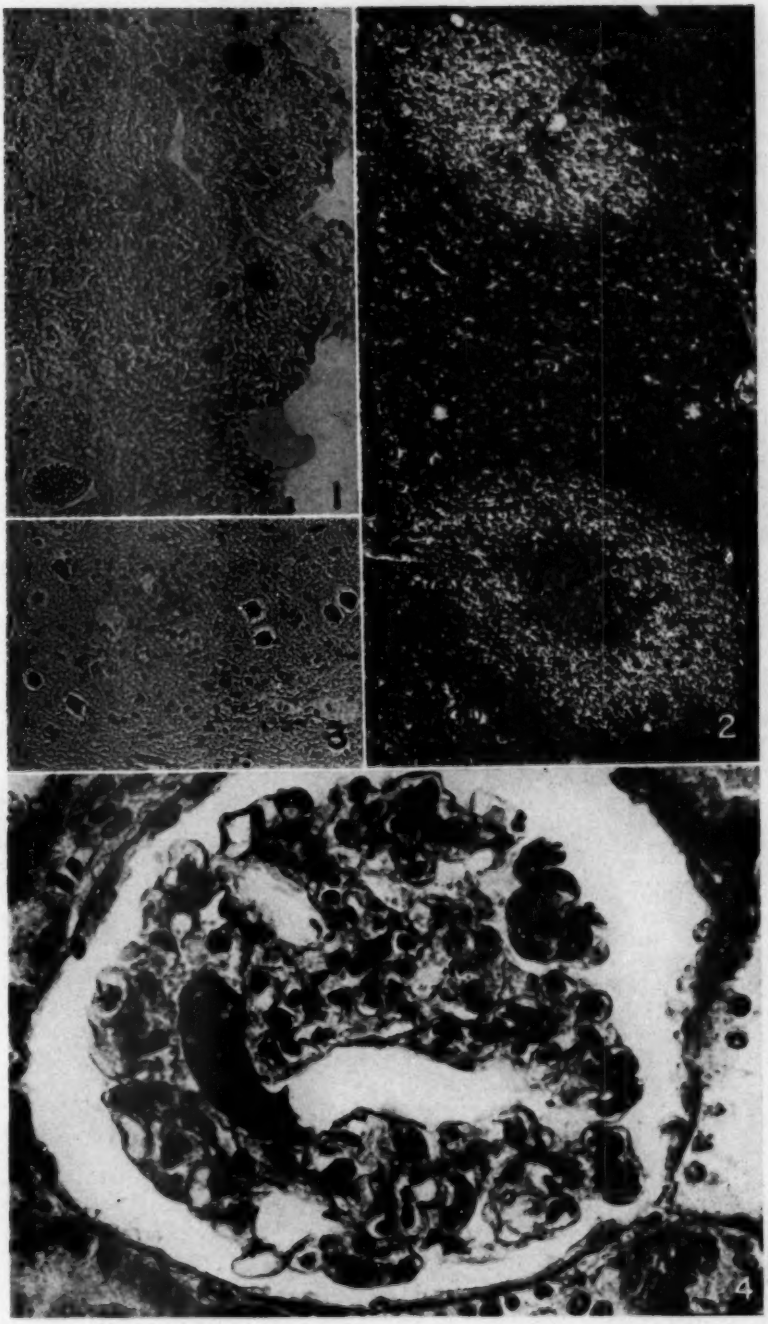

Fig. 1 (Case 1).—Fat emboli in sinusoids of the area postrema; floor of fourth ventricle. Sudan-stained, gelatine-embedded frozen section; × 170.

Fig. 2 (Case 1).—Ring hemorrhages of cerebral white matter, Phosphotungstic acid-hematoxylin-stained, formalin-fixed, paraffin-embedded material; × 150.

Fig. 3 (Case 1).—Focus of nerve cell loss and histiocytic proliferation in the inferior olivary nucleus. Thionine-stained, formalin-fixed, paraffin-embedded material; × 170.

Fig. 4 (Case 1).—Juxtaembolic fibrin in glomerular sinusoid of kidney; the empty lumen corresponds to the removed fat embolus. Phosphotungstic acid-hematoxylin-stained, formalin-fixed, paraffin-embedded material; × 625.

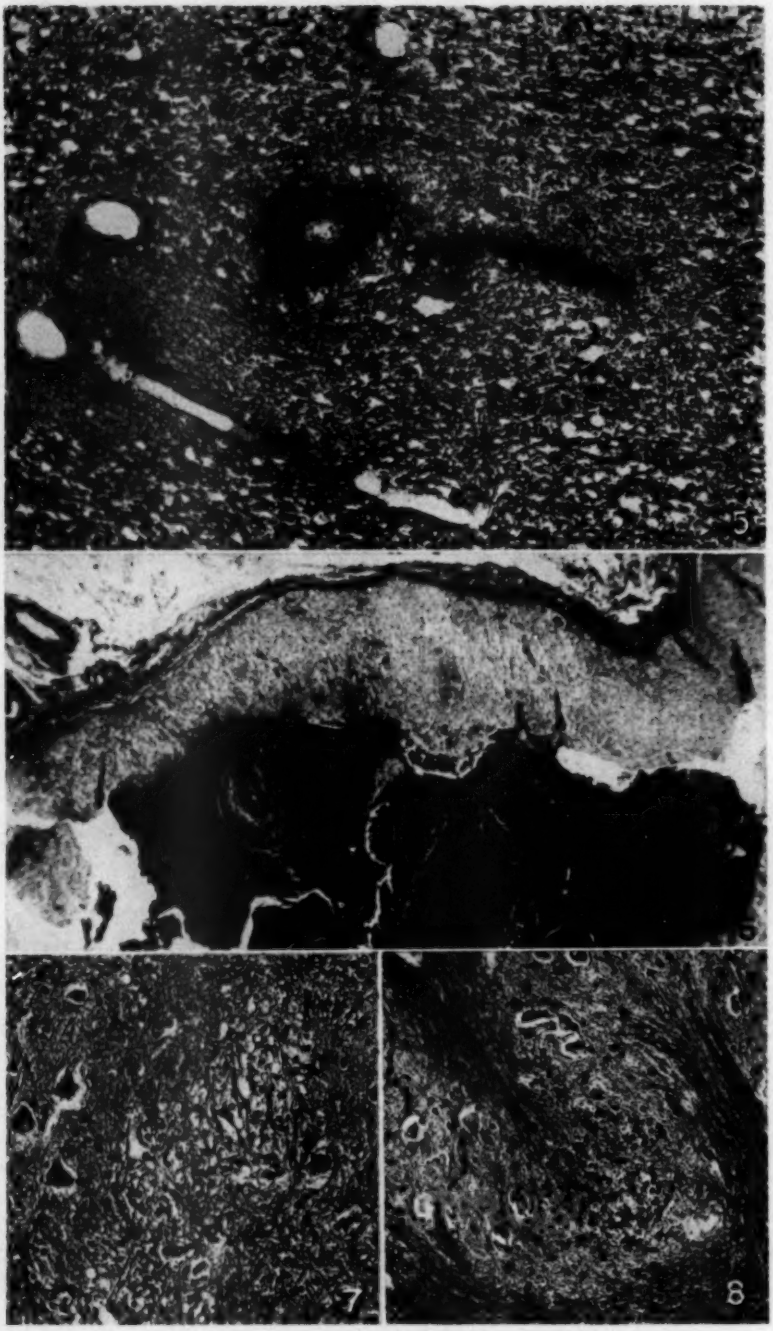

Fig. 5 (Case 1).—Juxtaembolic fibrin and fibrin deposited in perivascular space and tissue; the empty lumina correspond to the removed fat emboli. Phosphotungstic acid-hematoxylin-stained, formalin-fixed, paraffin-embedded material; × 143.

Fig. 6 (Case 1).—Massive hemorrhage in the medulla of the adrenal gland. Phosphotungstic acid-hematoxylin-stained, formalin-fixed, paraffin-embedded material; × 35.

Fig. 7 (Case 2).—Focus of nerve cell loss and histiocytic proliferation in dentate nucleus. Phosphotungstic acid-hematoxylin-stained, formalin-fixed, paraffin-embedded material; × 170.

Fig. 8 (Case 2).—Focus of nerve cell loss and histiocytic proliferation in the inferior olivary nucleus. Phosphotungstic acid-hematoxylin-stained, formalin-fixed, paraffin-embedded material; × 170.

of the brain stem, similar foci were profusely invaded by proliferating histiocytic elements (Fig. 3). In the white matter the minute necroses were surrounded by a zone of red blood cells, characteristic of ring hemorrhages (Fig. 2). Also, these necrotic foci were frequently invaded by microglial cells of histiocytic origin. Some of the latter histiocytes stained a faded homogeneous blue by the Prussian blue method. In addition minimal deposition of iron was demonstrable in the area postrema, the infundibulum, and the choroid plexus. Single cells with iron pigment were seen adjacent to vessels of these areas, which were free of degenerative changes. Phosphotungstic acid-hematoxylin-stained sections of kidney and brain presented fibrin both in the vessels and in the perivascular tissue. Scattered glomeruli were distinguished by the presence of dense fibrin masses of a deep purple hue (Fig. 4). The fibrin was attached to fat emboli which were removed during dehydration of the block for the paraffin embedding. In the brain, too, a few small central vessels of the white matter foci were occluded by fibrin adjacent to the emboli, i. e., in the center of ring hemorrhages. Threads of fibrin formed a purple network in many of the minute necrotic foci within the ring hemorrhages and around the central vessels. These complex findings were more conspicuous in large acutely infarcted areas of the white matter (Fig. 5). The vascular lumen adjacent to the segment occupied by the fat was occluded by large masses of fibrin; this contrasted with the embolized segment characterized by widely dilated empty lumina. Around the lumina was noted a perivascular formation of purple fibrin. Heavy deposits of fibrin obstructed some of the Virchow-Robin spaces. The amount of deposition varied greatly.

The medulla of the adrenal gland on one side was completely destroyed by a massive hemorrhage in which circular layers of fibrin had formed (Fig. 6). In several places the blood had perforated the cortex. Small isolated foci of cortical necrosis were present. Massive retroperitoneal hemorrhage was found along the ascending colon. Punctate hemorrhages occurred in the pericardium. Except for the recently repaired fracture of the leg, the remainder of the autopsy was not contributory.

CASE 2 (0.119/50).—*Clinical History.*—A 14-year-old girl had suffered for several years with polyarthritis deformans. During the last year she had experienced considerable improvement and less pains in her joints. With the improvement, the attending doctors were surprised to note that she had become somewhat obese. Her gait remained poor because of contractures of muscles of thigh and leg, and an operative stretching of the knee joints was advised. After the operation, which was uneventful, the patient showed symptoms of respiratory distress and cyanosis. She lay quietly in bed, apparently alert, although somewhat dizzy and without normal apprehension. No complete neurological examination was performed because the attending doctors were unaware of the severity of the condition. The patient died unexpectedly 39 hours after the operation.

Postmortem Examination.—Sudan-stained frozen sections contained numerous fat emboli in the lungs, the heart, and the kidney. A considerable number of emboli were found within the cerebral cortex, lenticular nuclei, thalami, dentate nuclei, and inferior olivary nuclei. In the last two regions the emboli were in several small foci of nerve cell degeneration. The foci displayed striking proliferations of histiocytes (Figs. 7 and 8). The cerebral white matter had only an occasional ring hemorrhage around the fat-embolized vessel. No fibrin thrombi were demon-

strated in any sections of the brain and kidney. The liver showed a severe degree of fatty infiltration which extended over the entire lobules. The remainder of the autopsy was not contributory.

COMMENT

Changes of Diagnostic Value.—The pathological effect of fat emboli becomes clinically evident when symptoms of respiratory distress with cyanosis develop (Killian,[2] Frey [3]) or when there is generalized vascular involvement with petechial hemorrhages (Benestad [4]) or cerebral damage with coma (Ribbert,[5] Payr,[6] Grøndahl [7]). Gross examination of the brain may support the clinical impression when innumerable petechial hemorrhages are seen in the white matter, i. e., so-called brain purpura (Schmidt [8]). The petechial hemorrhages are not specific for fat embolism and may sometimes be absent, as in Case 2 of this study. The unequivocal diagnosis of fat embolism rests on the demonstration of sudanophilic material in the vessels. The small round foci of necrosis which result are no doubt related directly to the clinical symptoms and the course of the disease. The cortex may be pierced by areas of cellular rarefaction (Gauss [9]), and as many as two-thirds of these areas may be typically located in the fifth and sixth cortical layers (Cammermeyer [10]). The number of lesions varies considerably. The brain stem often has degenerative foci as large as those seen in the cortex, but they are usually invaded by microglia (Weimann [11]). In the inferior olivary nuclei of a previously studied case, 29 hours sufficed to reproduce changes similar to those of the present two cases (Cammermeyer [10]). From these observations it is concluded that the cerebral changes in the two cases were caused by generalized embolization, which in Case 1 took place simultaneously with the onset of coma, about 46 hours prior to death, and in Case 2, during the operation, 39 hours prior to death. Definitive symptoms and signs of cerebral involvement would probably have been noted if a complete neurological examination had been performed in Case 2.

Pathophysiological Considerations.—The origin of the fat emboli is clear since the two patients had sustained fractures, and there was probably access to the bone marrow via ruptured veins, but the exact reasons for the ill effect of fat emboli is not obvious. In fact, several observations indicate that the mechanism concerned with the production and manifestation of fat emboli are rather complex

2. Killian, H.: Die traumatische Fettembolie, Deutsche Ztschr. Chir. **231**:97-186, 1931.

3. Frey, S.: Die Embolie, Leipzig, Georg Thieme, 1933.

4. Benestad, G.: Drei Fälle von Fettembolie mit punktförmigen Blutungen in der Haut, Deutsche Ztschr. Chir. **112**:194-205, 1911.

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7. Grøndahl, N. B.: Untersuchungen über Fettembolie, Deutsche Ztschr. Chir. **111**:56-124, 1911.

8. Schmidt, M. B.: Über Gehirnpurpura und hämorrhagische Encephalitis, Beitr. path. Anat. (Supp.) **7**:419-455, 1905.

9. Gauss, H.: Studies in Cerebral Fat Embolism, with Reference to the Pathology of Delirium and Coma, Arch. Int. Med. **18**:76-102, 1916.

10. Cammermeyer, J.: Cerebral Changes in an Acute Case of Fat-Embolism, Acta psychiat. et neurol. **12**:333-354, 1937.

11. Weimann, W.: Besondere Hirnbefunde bei cerebraler Fettembolie, Ztschr. ges. Neurol. u. Psychiat. **120**:68-83, 1929.

(Groskloss [12]). Gauss [9] noted fatty liver in a large number of fatal cases of posttraumatic fat embolism. Killian [2] indicated that the liver may be an important source of the fat. A disturbed fat metabolism predisposing to fatal fat embolism may account for such unexpected incedences in both obese patients and patients with fatty liver following arsphenamine (salvarsan) treatment (Burns and Bromberg [13]) and following exposure to simulated high altitudes (Haymaker and Davison [14]). This view is favored by the exceptional occurence of myocardial fat embolism in a case of severe centrolobular fatty liver, the source being related to the spontaneous rupture of fat-filled liver cells (Cammermeyer and Gjessing [15]). The exact relationship between disturbed fat metabolism, fat infiltration of visceral organs, and the sudden lack of balance of blood fat is not solved, but some mechanism of this kind is probably involved in Case 2. The extreme degree of fatty liver is taken as the morphological evidence of abnormal fat metabolism contributing to the fatal outcome. Otherwise in healthy youngsters the same surgical procedure is not complicated by fat embolism, i. e., no clinical manifestation of such embolization is noted.

Histopathological Considerations.—The presence of iron in histiocytes within the areas of central necrosis in the ring hemorrhages in Case 1 was not expected because of the short duration of the brain damage. According to general pathological experience the phagocytosis of iron pigment, which is derived from degenerating hemoglobin, cannot be demonstrated by the Prussian blue method earlier than four days after the onset of the pathological process. In an attempt to indicate another source of iron pigment it is postulated that leakage of diffusible compounds of serum iron through damaged walls of vessels could be a valuable source. The vascular alteration could be due to emboli either passing by or sticking to the wall of the vessel (Broman,[16] Swank and Hain [17]). The finding of perivascular iron in human patients would therefore have the same significance as the demonstration of an increased vascular permeability to trypan blue in experimental animals, the two being morphological evidence of pathological alteration of the vascular permeability. A single transfusion of blood was given to the patient in Case 1 two days prior to death, and this may have increased the concentration of serum iron. Further support that diffiusion of iron pigment was the source of the siderosis in the small areas of necrosis in the ring hemorrhages is to be found in the small amounts of iron stored in the infundibulum, the area postrema, and the choroid plexus. The three regions in question are characterized by a vascular system

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13. Burns, E. L., and Bromberg, L.: Fatal Multiple Fat Embolism in a Patient Given Salvarsan, Am. J. Syph. **14**:43-54, 1930.

14. Haymaker, W., and Davison, C.: Fatalities Resulting from Exposure to Simulated High Altitudes in Decompression Chambers: Clinicopathologic Study of 5 Cases, J. Neuropath. & Exper. Neurol. **9**:29-59, 1950.

15. Cammermeyer, J., and Gjessing, R.: Fatal Myocardial Fat-Embolism in Periodic Catatonia with Fatty Liver, Acta med. scandinav. **139**:358-367, 1951.

16. Broman, T.: Über cerebrale Zirkulationsstörungen: Tierexperimentelle Untersuchungen über Mikroembolien, Schädigungen der Gefässpermeabilität und Blutungen verschiedener Art, Acta path. et microbiol. scandinav., Supp 42, p. 1, 1940.

17. Swank, R. L., and Hain, R. F.: The Effect of Different Sized Emboli on the Vascular System and Parenchyma of the Brain, J. Neuropath. & Exper. Neurol. **11**:280-299, 1952.

which is permeable to acid semicolloidal dyes in experimental animals (Wislocki and King [18]) and to diffusible iron compounds of the blood in human hemochromatosis (Cammermeyer [19]). They are normally free of iron.

The unique occlusion of vessels by both fat and fibrin was noted in the kidney by Waaler [1]. The juxtaembolic formation of fibrin is encountered occasionally in the brain, too, in addition to the perivascular deposition of fibrin. For each of the three conditions of fibrin deposition the following mechanism is proposed.

(*a*) Juxtaembolic Fibrin in Areas of Cerebral Necrosis: It is known that brain contains yields rich in thromboplastic substances which may eventually be released after disintegration of cell boundaries. In Case 1 necrotizing lesions in both brain and adrenal glands may have served as significant sources of this material. Tromboplastin, which is soluable in water (Quick [20]), together with liquefied tissues could be dissolved and thus transported toward the vessels. Therefore, some of the thromboplastic material is expected to be absorbed by the thin-walled vessels into the venous blood. The latter phase of flow of thromboplastin initiates the formation of fibrin intravascularly, but in order to reach this location the rate of absorption is a decisive factor, with an optimal time ranging from two to five days. The fact that the fibrin is not demonstrated in cases of shorter duration, as in Case 2 and in the cases of sudden death, excludes a so-called postmortem nature of blood clotting. A direct effect of fat on the blood clotting mechanism is less plausible; otherwise one would expect to see the thrombus in all the embolized vessels and not in only a few of them (contrary to ideas of Waaler [1]).

(*b*) Juxtaembolic Fibrin in Remote Organs: The thromboplastic material absorbed from the multiple necrotic foci of various organs is responsible for the local formation of intravascular fibrin; however, some of it may enter the systemic blood circulation and be kept inactive by dilution and by neutralizing agents. Whenever the active agents become relatively concentrated, thrombi may be produced at remote venous locations, such as in the glomeruli of the kidney. This view receives some support from the occurrence of similar fibrin changes in both kidney and the choroid plexus in cases of septicemia with bilateral hemorrhagic infarction of the adrenal glands. In these cases the source of the liberated thromboplastic material is the acutely degenerated adrenals. The hemoconcentration following the process of filtration could account for the fibrin formation in the veins of the two regions. Only cases of delayed fatality may show such thromboemboli in the kidney (Waaler [1]; Case 1).

(*c*) Perivascular Deposition of Fibrin: The minute necrotic foci of white matter are frequently pierced by a network of fibrin, a well-known phenomenon

18. Wislocki, G. B., and King, L. S.: The Permeability of the Hypophysis and the Hypothalamus to Vital Dyes, with a Study of the Hypophyseal Vascular Supply, Am. J. Anat. **58**:421-472, 1936.

19. Cammermeyer, J.: The Area Postrema: A Contribution to Its Normal and Pathological Anatomy, Especially in Haemochromatosis, Norske Videnskaps-Akademi i Oslo, Skrifter, Matematisk-naturvidenskapelig klasse, 1944, No. 12, Oslo, Jacob Dybwad, 1945; Deposition of Iron in Paraventricular Areas of the Human Brain in Hemochromatosis, J. Neuropath. & Exper. Neurol. **6**:111-127, 1947.

20. Quick, A. J.: On Various Properties of Thromboplastin (Aqueous Tissue Extracts), Am. J. Physiol. **114**:282-296, 1936.

in instances of cerebral purpura (Wolff[21]). This fibrin has exactly the same staining qualities as the intravascular fibrin, i. e., pink with the hematoxylin and eosin method and deep purple with the phosphotungstic acid-hematoxylin method. The fibrin occurs in the two localities simultaneously. The presence of fibrin in the nervous tissue, which is free of collagen, eliminates the theoretical possibility that it is due to fibrinoid degeneration of connective tissue. An adequate explanation for the formation of fibrin may therefore be found in the previously discussed vascular damage with leakage of plasma constituents and in the concomitant necrosis of cells with release of thromboplastic material. The mixture of active constituents from the two sources would initiate formation of fibrin. It seems, therefore, to depend on the rate of perivascular flow of thromboplastic material and plasma constituents whether fibrin is noted in the perivascular tissue or in the perivascular space. The process of diffusion is an essential factor, but the necrosis of cells must be completed so that reaction takes place.

From the present observation the conclusion is reached that several factors are operating in the production of fibrin (tissue factors with liberation of thromboplastic material, hematogenous factors with exudation of plasma compounds, hemodynamic factors responsible for the introduction of thromboplastic material into the circulating blood and its subsequent concentration). The site of deposition of fibrin would seem to depend on the magnitude of each of these three factors.

CONCLUSION AND SUMMARY

The following are the pertinent findings in two different cases of cerebral fat embolism.

In a 47-year-old man (Case 1) the minute central necrosis of cerebral ring hemorrhages contained iron. The lapse of 46 hours, which is the duration of cerebral involvement with coma, is considered too short a time to allow for its production from degenerating hemoglobin. The iron is believed to be exuded from circulating serum iron through damaged walls of vessels.

The unique occurrence of fibrin adjacent to fat emboli in cerebral infarcts and in renal glomeruli is related to released thromboplastic material which in the lapse of three days (77 hours) is absorbed by the veins. A similar mixture of those substances may take place in the perivascular tissue and spaces when plasma constituents are permeated through the wall of vessels which are damaged by emboli.

In a 14-year-old child (Case 2) a striking fatty liver is taken as evidence of an abnormal fat metabolism. This condition had probably lowered the resistance to circulating fat emboli and then predisposed for the manifestation of fat embolism, which is rare at this age. The absence of fibrin in tissues and vessels is explained by the short clinical course of 39 hours; thereby no absorption of thromboplastic material was possible.

Dr. Roy L. Swank, of Montreal, Canada, revised the manuscript, and the Armed Forces Institute of Pathology, Washington, D. C., provided the photomicrographs.

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EFFECT OF AUREOMYCIN ON EXPERIMENTAL ARTERIOSCLEROSIS AND SERUM CHOLESTEROL LEVELS

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IN MANY studies on the production of experimental cholesterol arteriosclerosis in rabbits, the feeding of cholesterol has been combined with a variety of procedures designed to exaggerate or alleviate the resultant vascular lesions. Since aureomycin has been observed to exert a profound effect in the nutrition of a variety of animal species, the present experiments combined cholesterol feeding with oral administration of aureomycin.

MATERIAL AND METHODS

Twenty immature female rabbits, with an average weight of 1.9 kg., were divided into four groups. Groups 1 and 3 received 0.5% of cholesterol with their food, and Groups 2 and 4 received 1.0%. The food was finely ground Rockland rabbit pellets.[1] The cholesterol (Armour Laboratories) was powdered to sift through a 60-mesh screen in order to facilitate absorption without the addition of fat to the diet.[2] In addition to cholesterol, the third and fourth groups received aureomycin hydrochloride (Lederle Laboratories) as 0.025% of the diet. These rations and tap water were fed ad libitum. A small amount of hay was also given to the rabbits daily.

The animals were weighed every 4 weeks, and at the end of 20 weeks they were sacrificed by injection of air into an ear vein. Immediately thereafter blood was obtained from the jugular vein for chemical analysis.

The animals were examined for gross pathologic changes, and the following organs and tissues were fixed in formalin and embedded in paraffin for histologic examination: brain, pituitary

This investigation was supported in part by a Grant-in-Aid from the Lederle Laboratories Division of the American Cyanamide Company.

From the Department of Clinical Science, University of Illinois (Dr. Nelson and Dr. Ivy), the Department of Anatomy, University of Saskatchewan (Dr. Altschul), and the Hektoen Institute of Cook County Hospital (Dr. Willheim).

1. The average analysis reported by the Rockland Farms for their rabbit pellets gives the following percentage composition: protein 18.62, fat 2.65, fiber 13.70, carbohydrates 61.66, ash 6.56. The mineral composition (per cent) is SiO_2 1.11, Cl 0.16, Na 0.06, K 1.52, Ca 1.04, P 0.58, S 0.21, Mg 0.32; (milligrams per 100 gm.) Mn 4.90, Fe 36.00, Zn 2.60, Cn 0.25, B 2.45, Co 0.023, I 1.20, F trace. Vitamin content (micrograms per 100 gm.) is B_1 436 to 510, B_2 424 to 722, B_6 486 to 617, pantothenic acid 2,760 to 3,790, para-aminobenzoic acid 93; (milligrams) choline equivalent 712 to 748, inositol 36, niacin 18.4, alpha tocopherol 6, carotene 1.46 to 291; (international units) A 1,720 to 1,880, D 21, C trace.

2. Popjak, G.: The Effect of Feeding Cholesterol Without Fat on the Plasma-Lipids of Rabbit: Role of Cholesterol in Fat Metabolism, Biochem. J. **40**:608, 1946.

gland, eyes, tongue, thyroid gland, heart, aorta, skeletal muscle, thymus, lungs, parts of the gastrointestinal tract, liver, spleen, pancreas, kidneys, suprarenals, and ovaries. The sections were prepared in the usual way for microscopic examination.

The following chemical determinations were made on the blood serum: free and total cholesterol by the Chaney modification of the Sperry-Schoenheimer method [3]; total serum lipid by a turbidimetric method of de la Huerga and Popper [4]; inorganic phosphate by the method of Fiske and Subbarow [5]; nonprotein nitrogen, by a slight modification of the Folin-Wu technique,[6] using for deproteinization 10% trichloracetic acid, and lipid phosphorus by the method of Youngburg and Youngburg.[7] The liver fat was determined by drying the tissue with anhydrous sodium sulfate and extracting it with chloroform in the Soxhlet apparatus, followed by a second extraction with petroleum ether.

The histologic studies were made by one of us (Dr. Altschul), who was given the tissues without information regarding the group to which each animal belonged. The morphologic changes will not be described since they did not differ from those already known.

Vascular and parenchymal damage was assessed in each animal on the basis of the intensity, as well as the extent, of the topographic distribution of the lesions. It is well known that various types of tissue have a different affinity for cholesterol deposition, which, in the case of experimental cholesterol arteriosclerosis, may become extensive to such a degree that the cells suffer and even deteriorate. The epithelial cells of the suprarenal cortex, especially those of the zona fasciculata, are the first to show microscopically an accumulation of lipoids, followed by liver cells and much later, if the cholesterol intake has been maintained, by cells of the renal tubules or by other epithelial cells elsewhere. Only when the damage is very marked does skeletal muscle or smooth muscle show intrafibrous lipoid accumulations.

With regard to connective tissue, usually that of the ciliary body, then that of the iris, followed by the rest of the choroid of the eye, shows intracellular and extracellular lipoid deposits. Still later, deposits form in the intertubular connective tissue of the kidney and in the loose tissue of the choroid plexus. All these changes occur only after the reaction of the suprarenal cortex has become evident. In other regions of the body the connective tissue reaction will appear somewhat later. It should be understood, however, that it is not yet established how many of the foam cells are sessile or immigrated histiocytes and how many are foamy endothelial cells or their "descendents."

Similarly, the vessels have a different susceptibility for cholesterol arteriosclerosis. This discrepancy aids greatly in assessing the cholesterol damage. If the latter is not very intense, lesions will be restricted to the aorta, to lingual arteries, and to small branches of the coronary arteries. With increased intensity, the pulmonary vessels and subsequently the renal arteries will be affected, while mesenteric vessels, arterioles of the spleen and liver, and arterioles of skeletal muscle other than that of the tongue present changes if the cholesterol damage is very severe.[8]

3. Chaney, I. C.: Personal communication to the authors.

4. de la Huerga, J., and Popper, H.: Personal communication to the authors.

5. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**:375, 1925.

6. Folin, O., and Wu., H.: A System of Blood Analysis, J. Biol. Chem. **38**:81, 1919.

7. Youngburg, G. E., and Youngburg, M. V.: Phosphate Metabolism: A System of Blood Phosphorus Analysis, J. Lab. & Clin. Med. **16**:158, 1930.

8. Altschul, R.: Selected Studies on Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1950.

Naturally, the degree of the single lesion has also to be taken into account. It is usually not difficult to assess the height of foam cell layers in the aorta and in the larger pulmonary vessels or the thickness of lipoid cushions in coronary vessels. Thus, taking into consideration the involvement or the lack of involvement of the various vessels, of parenchymal tissues, and of the connective tissue, together with the gravity of the single lesions, the 20 cases were arranged into nine groups of different intensity, and a rating scale of 1 to 9 was therefore adopted.

We are aware that our method of evaluating the severity of cholesterol damage is arbitrary and that it depends on the subjective opinion of the investigator. However, a completely unprejudiced judgment can be made if the investigator making the assessment has no knowledge of the treatment received by the individual animals. In the present study it was deemed essential to have a quantitative measure of the morphological changes in each animal in order to determine their relationship to the chemical data.

RESULTS

At the beginning of the experiment the animals ate each day about 120 gm. of the powdered food, and they ate about 160 gm. during the last half of the period. This provided 30 to 40 mg. of aureomycin in the diets containing this supplement. The total aureomycin ingested by each rabbit was between 4.2 and 5.6 gm. The four groups ate approximately the same amount of food and gained weight at about the same rate.

When the scores for histological rating were averaged for each group, the following values were obtained: Group 1, receiving 0.5% cholesterol, 2.4; Group 2, receiving 1.0% cholesterol, 5.0; Group 3, receiving 0.5% cholesterol and 0.025% aureomycin, 4.4, and Group 4, receiving 1.0% cholesterol and 0.025% aureomycin, 8.8.

In Table 1 the data on initial and final body weight, rating of vascular and parenchymal injury, and the chemical analyses are presented as the average for each of the four groups of rabbits. The data were subjected to an analysis of variance.[9] We found no significant interaction of the variables. This permits combining the groups receiving 0.5% cholesterol and the group receiving 1.0% cholesterol, regardless of whether or not they were given aureomycin. So, too, the influence of the amount of cholesterol ingested can be disregarded in combining the 10 animals that received aureomycin and the 10 that did not. In Table 2 the same data are arranged to show the effect of adding aureomycin to the diet by combining the values for Groups 1 and 2 and comparing their means with those of Groups 3 and 4. Table 2 also demonstrates the effect of raising the cholesterol from 0.5 to 1.0% of the diet by contrasting the means of Groups 1 and 3 with those of Groups 2 and 4.

Analysis of variance [9] allows us to conclude that increasing the cholesterol in the diet from 0.5% to 1.0% significantly increases the cholesterol, total lipid, and lipid phosphorus levels of the serum and the cholesterol/phospholipid ratios. The amount of fat in the liver and the vascular and parenchymal injury are also significantly increased.

9. Snedecor, G. W.: Statistical Methods Applied to Experiments in Agriculture and Biology, Ed. 4, Ames, Iowa, The Collegiate Press, Inc., 1946.

The addition of 0.025% of aureomycin to the diet produced statistically significant increases in the estimate of vascular injury, in the cholesterol and total lipid of the serum, and in the cholesterol/phospholipid ratio. The percent of cholesterol esterified was decreased.

TABLE 1.—*Effect of Dietary Supplements of 0.5% and 1.0% of Cholesterol, With and Without Addition of 0.025% of Aureomycin* *

	Group 1 (Given 0.5% Cholesterol)		Group 2 (Given 1% Cholesterol)		Group 3 (Given 0.5% Cholesterol Plus Aureomycin)		Group 4 (Given 1% Cholesterol Plus Aureomycin)	
	Mean	S. E.	Mean	S. E.	Mean	S. E.	Mean	S. E.
Initial body weight, kg.	2.0	0.09	1.9	0.18	1.8	0.18	1.9	0.04
Final body weight, kg.	5.4	0.22	5.0	0.22	4.6	0.49	5.2	0.18
Estimate of vascular and parenchymal injury†	2.4	0.72	5.0	0.72	4.4	1.07	8.8	0.04
Liver fat, %	3.5	0.22	4.1	0.49	3.1	0.26	5.1	0.54
Serum								
Total cholesterol, mg. per 100 cc.	220.0	46.06	669.0	136.42	521.0	92.12	1,034.0	122.99
Cholesterol esterified, %	67.8	4.35	71.2	4.25	51.6	7.15	63.1	4.40
Lipid phosphorus,‡ mg. per 100 cc.	10.6	0.35	16.0	2.25	12.2	1.77	19.9	1.73
Cholesterol/phospholipids§	30.0	3.54	38.0	4.44	40.0	10.54	54.0	2.09
Total lipid, mg. per 100 cc.	873.0	50.53	1,379.0	143.11	1,334.0	173.97	1,666.0	58.14
Nonprotein nitrogen, mg. per 100 cc.	60.1	3.62	57.8	3.71	61.5	5.28	63.7	1.25
Phosphate, mg. per 100 cc.	5.9	0.40	5.7	0.54	5.4	0.40	5.3	0.40

* Each group represents five rabbits.
† Estimate is based on nine groups of different intensity (rating scale, one to nine).
‡ The lipid phosphorus was determined for only 10 of the 20 animals, which were distributed as follows: two each in Groups 1 and 3 and three each in Groups 2 and 4.
§ The cholesterol/phospholipid ratios are based on 10 animals.

TABLE 2.—*Combined Groups Showing the Influence of Aureomycin and of Increasing the Level of Dietary Cholesterol* *

	Groups 1 and 2 (No Aureomycin)		Groups 3 and 4 (Given 0.025% Aureomycin)		Groups 1 and 3 (Given 0.5% Cholesterol)		Groups 2 and 4 (Given 1% Cholesterol)	
	Mean	S. E.	Mean	S. E.	Mean	S. E.	Mean	S. E.
Final body weight, kg.	5.2	0.16	4.9	0.25	5.0	0.28	5.1	0.13
Estimate of vascular and parenchymal injury†	3.7	0.63‡	6.6	1.23	3.4	0.70	6.9	1.14
Liver fat, %	3.8	0.28	4.2	0.57	3.3	0.22	4.7	0.41
Serum								
Total cholesterol, mg. per 100 cc.	494.0	121.12‡	778.0	102.15	421.0	59.14‡	852.0	95.19
Cholesterol esterified, %	69.5	2.97‡	57.4	4.77	59.7	5.20	67.2	3.25
Lipid phosphorus,§ mg. per 100 cc.	13.8	1.83	16.8	2.19	11.4	0.75‡	17.9	1.71
Cholesterol/phospholipid‖	35.0	3.49¶	48.0	4.96	34.0	5.35¶	43.0	4.16
Total lipid, mg. per 100 cc.	1,126.0	110.68¶	1,560.0	161.83	1,104.0	114.48‡	1,524.0	86.30
Nonprotein nitrogen, mg. per 100 cc.	59.0	2.47	62.6	2.56	60.8	3.10	60.8	1.96
Phosphate, mg. per 100 cc.	5.8	0.32	5.3	0.82	5.6	0.88	5.5	0.33

* The combined groups consist of 10 rabbits.
† Estimate is based on nine groups of different intensity (rating scale, 1 to 9).
‡ $p = < 0.01$. The p values are for the differences between the groups receiving or not receiving aureomycin and for the groups receiving different amounts of cholesterol.
§ The lipid phosphorus was determined for only 10 of the 20 animals. Means for five animals are 13.8 and 16.8; the mean for four animals is 11.4, and the mean for six animals is 17.9.
‖ The cholesterol/phospholipid ratios are based on 10 animals.
¶ $p = < 0.05$.

The following correlation coefficients were obtained on the 20 individual rabbits, regardless of treatment: estimate of vascular lesions and serum cholesterol +0.77, estimate of vascular lesion and serum lipid +0.79, serum cholesterol and total lipid +0.74. There was no significant correlation between any of the other values.

The low value for final body weight in Group 3 was due to loss of weight by one animal. On the 16th week of the experiment the average weight gain for the four groups was 2.19, 2.10, 2.18, and 2.24 kg., respectively. At that time one of the animals in Group 3 had chest râles. The animal lost weight during the last month of the experiment, but at autopsy no sign of infection was detected, and neither histologic nor chemical measures distinguish it from the rest of the group.

Aside from the vascular lesions, all of the rabbits in Group 1 (0.5% cholesterol) appeared to be in good condition. One rabbit in Group 2 (1% cholesterol) had several small fluid-containing cysts on the surface of the liver. This animal had the highest total serum lipid in the group but no other extremes. One animal in Group 3 (0.5% cholesterol and aureomycin) and one in Group 4 (1% cholesterol and aureomycin) had small areas of consolidation in the large lobes of the lung and had a slight ascites. The rabbit in Group 4 also had a small gastric ulcer. Both of these rabbits had the highest cholesterol levels in their respective groups.

COMMENT

The differences in the intensity and extent of vascular lesions can be explained in part by the different amounts of ingested cholesterol. The parallelism between the degree of injury and the quantity of cholesterol fed is more marked than in previous reports by other workers.

Of particular interest in the present investigation is the role of aureomycin, which enhanced the arteriosclerosis. The physiological activities and toxicity of aureomycin are not sufficiently known to allow an explanation, but the high correlation between vascular injury and serum cholesterol, together with the increased cholesterol/phospholipid ratio, suggests that whatever the mechanism involved, the lesions were precipitated by the serum changes.

The most probable physiological activity of aureomycin leading to hypercholesterolemia would seem to be its modification of the intestinal flora. This offers several possibilities; it might diminish the conversion of cholesterol to coprosterol, an inhibition which has been reported following the administration of succinylsulfathiazole [10]; thus additional cholesterol would presumably be available for absorption.

An equally tenable explanation would be a change in flora leading to an increase in the availability to the host of lipotropic substances, such as choline or vitamin B_{12}. It has been demonstrated by de la Huerga and co-workers [11] that aureomycin inhibits the bacterial transformation of free choline to trimethylamine in the intestine. Baxter and co-workers report [12] an increase of tissue choline in aureomycin-treated choline-deficient rats that is independent of food intake. Weiss and co-workers [13] have shown that in cholesterol-fed rats choline and other lipotropic

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13. Weiss, S. B.; Marx, L., and Marx, W.: The Effects of Thyroid and of Choline and Inositol on Cholesterol Distribution in Rats Fed a High Cholesterol Diet, Endocrinology **50**:192, 1952.

substances increase the serum cholesterol. This is also true in chickens.[14] Ridout and co-workers [15] found an accelerated absorption of cholesterol if adequate choline is administered simultaneously, and Wissler and co-workers [16] observed that atheromatous lesions are more frequent in the coronary arteries of cholesterol-fed rats if their diets are high in choline.

The serum lipid changes in our rabbits might also have occurred as a result of trauma to the liver. Lesions and the deposition of fat in the liver have been described by Lepper and co-workers [17] following the administration of excessive amounts of aureomycin to patients and experimental animals. In our animals the liver lesions observed on histologic examination were attributable to the cholesterol. The 30 to 40 mg. of aureomycin ingested daily by our rabbits is below the usual therapeutic dose; however, it is possible that aureomycin given over a period of 20 weeks might lead to functional hepatic changes which we did not measure.

Although there was a statistically significant decrease in the esterification of cholesterol in our aureomycin-fed animals, we do not feel confident in ascribing this to liver injury, because the value obtained for the group in question was heavily weighted by one extremely low figure.

Application of Bartlett's test of homogeniety of variance [18] showed this series of determinations to be lacking in homogeniety, which invalidates the statistical evaluation.

The increase in total serum cholesterol and cholesterol/phospholipid ratio may not be the only relevant changes brought about by the prolonged administration of aureomycin. We cannot rule out the possibility that aureomycin has a direct action on the vascular system, damaging the endothelium proper, and thus facilitating atheromatosis.

SUMMARY

Raising the cholesterol content of the diet from 0.5% to 1.0% increases the severity and extent of vascular and parenchymal lesions in rabbits. In this experiment significant increases were also observed in the cholesterol and lipid levels of the serum and in the cholesterol/phospholipid ratio. The liver fat was also increased.

The addition of 0.025% of aureomycin to these diets further augmented all of these manifestations of cholesterol toxicity, except the amount of fat in the liver, which did not differ significantly from that of the rabbits fed cholesterol without aureomycin.

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EXPERIMENTAL ENDOCARDITIS IN NORMAL ANIMALS AND IN ANIMALS TREATED WITH CORTISONE

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MINNEAPOLIS

IN PREVIOUS experiments it was found that endocarditis could be produced rather uniformly in rats [1] by giving intracardiac injections of streptococci of either the viridans or the hemolytic group and in rabbits [2] by giving intravenous injections of the viridans group.

In the rats the anatomic characteristics of the vegetations on the valve seen by both gross and microscopic examination conformed fairly closely to a uniform pattern in the different animals. In some of the vegetations the picture was that of typical bacterial endocarditis, while in others the appearance was more closely that seen in human acute rheumatic endocarditis. The two types of vegetations were commonly found together on the same valve, but at other times a single kind was chiefly observed. In the rabbit [3] the endocarditis resembled more closely the bacterial type of endocarditis in human patients. However, in some rabbits, lesions having a microscopic structure much like human rheumatic endocarditis were seen. The animals, both rats and rabbits, developed a fairly high concentration of circulating antibodies (agglutinins).

This study seems to have some importance, since the endocarditis produced experimentally, especially in rats, approximates the anatomic picture of human acute rheumatic endocarditis more closely than any experimental cardiac lesions yet found. An experimental basis is provided for studying the effects of cortisone upon cardiac lesions, some of which anatomically resemble acute rheumatic endocarditis.

Treatment with cortisone has been reported to have beneficial effects in patients with acute rheumatic fever, in which endocarditis occurs so frequently.[4] The rat is an animal in which hypersensitivity to the Streptococcus, the organism used in these experiments, cannot be developed,[1] while in the rabbit hypersensitivity

From the Department of Pathology, University of Minnesota.

This study was aided by a grant from the United States Public Health Service.

Cortisone was supplied by Merck & Company for some of the earlier experiments.

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3. Clawson, B. J., and Bell, E. T.: A Comparison of Acute Rheumatic and Subacute Bacterial Endocarditis, Arch. Int. Med. **37**:66, 1926.

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(delayed type) to streptococci can easily be produced.[5] It was of interest to see if there were any suggestions that a potential hypersensitivity had any significant part in the pathogenesis of experimental endocarditis.

The effect of cortisone treatment upon experimental tissue response and the development of various immune reactions, such as antibody formation, anaphylactic reactions (immediate hypersensitivity), and bacterial reactions (delayed hypersensitivity), have been found to vary in the hands of different investigators. The connective tissue elements have been reported as being suppressed,[6] but, on the other hand, no changes have been noted.[7] Antibodies have been described as not being affected[8] or as being reduced by cortisone treatment.[9] Hypersensitivity,

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immediate or delayed, has been described as not being affected,[10] while in other reports it is described as being reduced.[11]

In the experiments reported in this paper the rat and the rabbit were the animals used for the following reasons: Endocarditis had previously been produced in these animals. Circulating antibodies (agglutinins) were also developed in fairly high concentrations in these animals. Hypersensitivity of either the anaphylactic or the bacterial type could not be produced in the rat.[1] The findings in regard to hypersensitivity in the rat agree with the observations of Hehre and Freund.[12] The rabbit, on the other hand, could be made to develop the bacterial (delayed) type of hypersensitivity to streptococci.[5]

The purpose of the experiments, therefore, is to find if cortisone alters the incidence, the gross and microscopic anatomic characteristics of the endocarditis produced, or the concentration of circulating antibodies during the process of developing the endocarditis in an animal, the rat, which does not become hypersensitive to the inciting agent of the endocarditis and in an animal, the rabbit, in which delayed hypersensitivity can easily be produced. It was thought that possibly some information might be obtained concerning any significance of the presence or absence of hypersensitivity in the pathogenesis of endocarditis.

EXPERIMENTS

The endocarditis, as in earlier experiments, was produced by injecting 0.5 cc. of a 48-hour broth culture of Streptococcus into the ventricular cavity, generally the left, of the rat (two injections at five-day intervals). The rabbits were given injections in a similar manner but with 5 cc. of the broth culture. If the animals did not die they were killed five days after the last injection. One strain of Streptococcus was used. It had been isolated from the blood of a

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11. Germuth, F. G.; Oyama, J., and Ottinger, B.: The Mechanism of Action of 17-Hydroxy-11-Dehydrocorticosterone (Compound E) and of the Adrenocorticotropic Hormone in Experimental Hypersensitivity in Rabbits, J. Exper. Med. **94**:139, 1951. Harris, S., and Harris, T. N.: Effect of Cortisone on Some Reactions of Hypersensitivity in Laboratory Animals, Proc. Soc. Exper. Biol. & Med. **74**:186, 1950. Nelson, C. T.; Fox, C. L., Jr., and Freeman, E. B.: Inhibitory Effect of Cortisone on Anaphylaxis in the Mouse, ibid. **75**:181, 1950. Long, J. B., and Favour, C. B.: The Ability of ACTH and Cortisone to Alter Delayed Type of Bacterial Hypersensitivity, Bull. Johns Hopkins Hosp. **87**:186, 1950. Germuth, F. G., Jr.; Nedzel, G. A.; Ottinger, D., and Oyama, J.: Anatomic and Histologic Changes in Rabbits with Experimental Hypersensitivity Treated with Compound E and ACTH, Proc. Soc. Exper. Biol. & Med. **76**: 177, 1951. Cohen, S. G., and Moses, C.: Effect of Cortisone on Experimental Production of Arteritis by Passive Sensitization, J. Lab. & Clin. Med. **37**:764, 1951. Footnote 6*d*. Footnotes 8*b* and *c*. Footnote 9*d*.

12. Hehre, E., and Freund, J.: Sensitization, Antibody Formation and Lesions Produced by Tubercle Bacilli in the Albino Rat, Arch. Path. **27**:289, 1939.

patient with acute rheumatic fever and had been used in the production of experimental endocarditis. This organism did not easily agglutinate spontaneously. It was, therefore, possible to perform tests for agglutination with a fair degree of accuracy.

In the agglutination tests the sediment of a several-day-old broth culture of Streptococcus was mixed in normal saline so that a faint milky suspension occurred. To 1 cc. of this suspension in different Wassermann tubes, 1 cc. of each of the various dilutions of the serums was added. These tubes were incubated for two hours at 40 C. in a water bath. They were then placed in a refrigerator at 2 to 4 C. for 24 hours and were then compared with controls. A maximum amount of definite agglutination was determined.

Rats weighing approximately 200, 100, or 50 gm. were used. The amount of cortisone acetate per dose given to each of these rats was 10.5 and 2.5 mg., respectively. This was approximately 1 mg. of cortisone acetate per 20 gm. of animal weight. It had been found that endocarditis developed with about the same incidence and character in animals of the above weights. It had also been found that the rat could stand such large daily doses for as long as 15 days, as a rule, without developing abscesses in the viscera. With a dose of 1 mg. of cortisone acetate per 10 gm. of animal weight, abscesses almost uniformly occurred, and occasionally they occurred when 1 mg. of cortisone acetate per 20 gm. of weight was used. Endocarditis never developed in 70 control rats which received cortisone only, even when abscesses developed in the rats which had received large doses and when streptococci were recovered from the abscesses in pure culture.

The weight of each rabbit was approximately 1,700 gm. Fifty milligrams of cortisone acetate was injected intramuscularly daily. This was 1 mg. per about 35 gm. of animal weight. In a series of 10 rabbits treated with cortisone only, no anatomic lesions developed.

All animals were pretreated with three daily injections of cortisone before the first streptococcic intracardiac injection and then were given injections daily with the same amount of cortisone until the day before they were killed. In some instances the animals died with endocarditis before the time for killing.

RESULTS

Endocarditis.—In previous studies with many rats endocarditis was never found to develop without streptococcic injections (intracardiac or intravenous). The incidence and gross characteristics of the endocarditis in the treated and nontreated rats are noted in Table 1. In the 100 treated animals endocarditis was found in 44(44%). In the 89 nontreated animals endocarditis occurred in 41 (46%).

The gross characteristics considered were the size of the vegetations and the valves or mural endocardium involved. Three sizes of vegetations (large, medium, and small) were recorded. The large size was almost great enough to fill the orifice. The small ones could be seen with some difficulty with the naked eye. The medium-sized vegetations were intermediate. It is noted that the percentage relationship of the sizes of the vegetations in the treated and nontreated groups is nearly the same. The mitral and aortic valves alone or in combination with other valves were the ones chiefly affected. All valves were involved in both treated and nontreated animals. A few cases of mural endocardial involvement were noted.

The microscopic characteristics studied in the valves were exudation of polymorphonuclear leucocytes, fibroblastic proliferation, fibrinoid formation, and the presence and relative numbers of bacteria in the vegetations (Table 2). These microscopic evaluations were made from a few sections through each valve and vegetation. Little variation in the percentage of the exudation of polymorphonuclear leucocytes is noted in the treated and nontreated animals. The degree of proliferation of fibroblasts is of interest, for on the basis of the findings of Ragan and others in a study of the connective element in rabbits treated with cortisone there is a general belief that the growth of connective tissue is retarded. Fibro-

blastic proliferation occurred in some degree in all of the treated and nontreated animals. In the severe degree the proliferation was somewhat greater in the treated animals.

The presence of fibrinoid is one of the most characteristic anatomic findings noted in the valves in acute rheumatic endocarditis. This fibrinoid reaction is also

TABLE 1.—*Incidence and Gross Characteristics of Endocarditis in Rats and Rabbits*

Incidence		Size of Vegetation			Valves or Ventricle Involved*							
No.	%		No.	%	M	A	T	P	MA	MT	MP	V
Treated rats (100)												
44	44.0	Large	7	15.9	37	3	1	0	1	0	1	1
		Medium	22	50.0								
		Small	15	34.0								
Nontreated rats (80)												
41	46.0	Large	8	19.5	34	1	2	1	0	1	0	2
		Medium	19	46.3								
		Small	14	34.1								
Treated rabbits (42)												
22	52.3	Large	9	40.9	6	0	7	0	0	7	0	2
		Medium	7	31.8								
		Small	6	27.2								
Nontreated rabbits (33)												
18	54.5	Large	2	11.1	9	1	5	0	0	1	0	2
		Medium	15	83.3								
		Small	1	5.5								

* M, mitral valve; A, aortic valve; T, tricuspid valve; P, pulmonary valve; MA, mitral and aortic valves; MT, mitral and tricuspid valves; MP, mitral and pulmonary valves; V, ventricle.

TABLE 2.—*Microscopic Characteristics of Endocarditis in Rats and Rabbits*

	Treated Rats (44)		Nontreated Rats (41)		Treated Rabbits (20)		Nontreated Rabbits (13)	
	No.	%	No.	%	No.	%	No.	%
Polymorphonuclear leucocytic exudation								
None	3	6.8	5	12.1	3	15.0	1	7.6
Moderate	30	68.1	27	65.8	8	40.0	11	84.6
Extensive	11	25.0	9	21.9	9	45.0	1	7.6
Fibroblastic proliferation								
None	0	0.0	0	0.0	1	5.0	0	0.0
Moderate	32	72.7	36	87.8	18	90.0	11	84.6
Extensive	12	27.2	5	12.1	1	5.0	2	15.3
Fibrinoid formation								
None	4	9.1	10	24.3	2	10.0	1	7.6
Moderate	36	81.8	25	60.9	15	75.0	8	61.5
Extensive	4	9.1	6	14.6	3	15.0	4	30.7
Bacteria								
None	33	75.0	31	75.6	13	65.0	9	69.2
Moderate	1	2.2	1	2.4	2	10.0	3	23.1
Extensive	10	22.7	9	21.9	5	25.0	1	7.6

noted in some degree in most cases of bacterial endocarditis. It was of interest to see if the treatment with cortisone caused a difference in the amount of fibrinoid in the treated and nontreated animals. Fibrinoid was noted in most of the two groups of rats but more commonly in the treated animals. In the smaller degree it was seen more frequently in the treated, but in the severe degree it was greater in the nontreated animals. These small differences appeared to be insignificant.

Bacteria (cocci) in variable amounts were observed generally in colonies in the platelet thrombi of the vegetations in 11 of the 44 treated animals (25%) and in 10 of the 41 nontreated animals (24.3%). It is likely that there would have been a higher percentage of positive findings of bacteria in the two groups if many serial sections had been examined.

Agglutination Titers in Treated and Nontreated Rats.—In the treated group of 29 animals, 16 had maximum titers of 1 to 3,200, and 13 had 1 to 6,400. In the 23 nontreated animals, 11 had titers of 1 to 3,200, and 12 had 1 to 6,400.

Endocarditis in Rabbits.—The incidence and gross characteristics of the endocarditis in the rabbits are seen in Table 1. Of the 42 treated rabbits studied, 22 had endocarditis (52.3%). Endocarditis was present in 18 of the nontreated rabbits (54.5%). A greater variation in the size of the vegetations and in valvular involvement, probably not significant, is noted in the two groups of rabbits rather than in the rats. The microscopic characteristics of the vegetations in the rabbit are tabulated in Table 2, where the structure of 20 treated rabbits and 13 nontreated rabbits is recorded. There is not as great uniformity of the polymorphonuclear leucocytic exudation in the treated and nontreated rabbits as in the rats. The fibroblastic proliferation, fibrinoid formation, and quantity of bacteria are nearly the same in the treated and nontreated rabbits.

Agglutination Titers in Treated and Nontreated Rabbits.—The serums of 15 treated rabbits were tested. All had titers of 1 to 6,400. In 15 serums of nontreated animals agglutination was seen in dilutions of 1 to 3,200 in 4 animals and in 1 to 6,400 in 11 animals.

COMMENT

Two immunological types of animals, the white rat and the rabbit, were studied in these experiments in respect to the effects of cortisone treatment upon experimental endocarditis and antibody formation.

A strain of the viridans group of streptococci was used in producing the endocarditis. It had previously been found impossible to produce hypersensitivity (delayed type) to this organism in the rat, while the rabbit was easily made hypersensitive.

Cortisone in large doses, as compared with recommended relative doses in human conditions, was administered subcutaneously or intramuscularly to the rat and intramuscularly to the rabbit. In a series of experiments equal results were obtained by either method with the above doses in the rat in respect to endocarditis, abscesses in the viscera, and antibodies. The animals were pretreated for three days before the intracardiac injection of streptococci and daily afterward until death or until the animals were killed.

No apparent significant difference in the incidence of endocarditis, gross characteristics (size of vegetations and valves or mural endocardium involved), or microscopic characteristics (polymorphonuclear leucocytic exudation, fibroblastic proliferation, occurrence of fibrinoid, and quantity of bacteria within the vegetations) was noted in the treated and nontreated rats and rabbits. Antibodies (agglutinins) were noted in practically the same dilutions in the treated and nontreated animals.

No useful or harmful indications for or against cortisone treatement were detected with these large doses given daily over a period of about two weeks in

respect to endocarditis and antibody formation following streptococcic injections in the above two types of animals.

It is of interest to note that proliferation of the connective tissue element in the valves is not suppressed in either animal. In this respect our findings differ from the observations of Ragan and others, who found a suppression of connective tissue in the ear of the rabbit under cortisone treatment.

While it has been found that cortisone may bring about desensitization in sensitized animals, nothing significant in our experiments suggested a different response in the valves under cortisone treatment in animals susceptible to bacterial hypersensitivity (rabbits) or in nonsusceptible animals (rats). There was only a slightly higher incidence of endocarditis in the potentially hypersensitive animal than in the rat in which bacterial hypersensitivity does not occur. There was no difference in the concentration of antibodies (agglutinins) in the two types of animals, and the development of antibodies was not suppressed.

SUMMARY

Cortisone given in large doses daily for two weeks does not alter significantly the incidence of experimental streptococcic endocarditis, the gross and microscopic characteristics of the endocarditis, or the concentration of circulating antibodies (aglutinins) in rats which are not susceptible to bacterial hypersensitivity or in rabbits which are susceptible.

OBSERVATIONS ON THYROID COLLOID

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IT HAS long been recognized that in histologic sections of the thyroid gland there often may be variations in the intensity of the staining of the colloid. Variations in the staining of colloid may also be noted in comparing sections obtained from thyroid glands representing various functional states. This may be appreciated by using such routine methods as hematoxylin and eosin or basic dyes such as thionine, methylene blue, or toluidine blue. Although there have been studies demonstrating the relationship of alterations of intracellular lipids,[1] intracellular colloid,[2] and enzymatic systems to various functional states, little has been written concerning the relationship of the follicular colloid to function. Similarly, the nature of the staining of colloid and the significance of the variations observed have received little attention. The purpose of this study is to attempt to explain some of these mechanisms by histochemical methods, as well as to report their relationship, if any, to thyroid function.

MATERIAL

Blocks of thyroid tissue from 27 persons exhibiting the morphologic and clinical features of various thyroid states were studied. These were obtained as surgical specimens.[3] There were five instances of diffuse follicular hyperplasia with moderate to severe lymphocytic infiltration. These glands were obtained from patients whose histories and laboratory findings, including basal metabolic rate, were consistent with a diagnosis of thyrotoxicosis. Five glands demonstrated moderate follicular hyperplasia and spur formation, as well as a lymphocytic infiltrate, and were from patients whose histories were indicative of thyrotoxicosis but who had received thiouracil, iodine, or both preoperatively. Four thyroids revealed a diffuse epithelial oxyphilia with indistinct or small follicles, slight distortion of thyroid plates, and varying degrees of lymphocytic infiltration and fibrosis. Histories from three of these patients revealed basal metabolic rates ranging from —10 to —18. These were considered examples of struma lymphomatosa. There were four instances of nodular goiter characterized by large and small follicles with marked distortion of the thyroid plates and varying degrees of fibrosis. Two glands revealed a similar histologic picture, but in addition some of the nodules revealed epithelial hyperplasia. Patients of these latter two groups complained only of thyroid enlargement. Sections from two patients demonstrated diffuse follicular enlargement with moderate distortion of the thyroid plates and no evidence of epithelial hyperplasia and were classified as diffuse colloid goiter. Five sections of thyroid were obtained from uninvolved areas of glands harboring a carcinoma or adenoma and were considered examples of normal thyroid.

From the National Institutes of Health, United States Public Health Service, Federal Security Agency.

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3. Many of these specimens were furnished through the courtesy of J. B. Hazard, M.D., Pathologist, Cleveland Clinic.

These studies also included sections of thyroid glands from five hypophysectomized Sprague-Dawley rats and the glands of three that had received thiourea; five that had been hypophysectomized, in addition to receiving thiourea, and five untreated controls.[4]

METHODS

All tissues were fixed in formalin. In addition, many blocks of human thyroid tissue were also placed in 95% alcohol and Zenker's acetic fluid. All tissues were fixed for approximately 16 hours, and tissues fixed in Zenker's acetic fluid were thoroughly washed in running water. All tissues were taken to water in the usual manner except on occasion when they were mounted on slides in alcohol prior to the removal of paraffin. Frozen sections were prepared from formalin-fixed tissue.

Staining Procedures.—The staining procedures consist of the following:

1. Eosin (0.5%) for two minutes, alcohol, xylene, resin.

2. Thionine (0.05%) in M/100 acetate buffer (pH 4) for 30 minutes.

3. Periodic acid-Schiff reaction; alum hematoxylin counterstain for two minutes.[5]

4. Ferric ferricyanide reduction test without counterstain.[6]

Blocking Procedures.—The blocking procedures were followed by all of the four staining procedures or by stains indicated.

Hydroxyl and Amine Blockade:

1. Acetylation with acetic anhydride-pyridine (16:24) for 24 hours at 25 to 30 C.[7] Controls for same interval in pure acetic anhydride and pure pyridine.

2. Benzoylation with benzoyl chloride-pyridine (2:38) for 4 hours at 58 to 60 C. Controls for same interval in benzoyl chloride alone and pure pyridine at 58 to 60 C.

Carboxyl and Other Acid Groups:

3. Methylation[8] with 0.05N HCl in methyl alcohol for 1, 2, 4, 6, 8, 16, 24, 48, and 72 hours at 58 C. and at 25 to 30 C. for 1, 2, 4, 6, 8, 16, 24, 48, and 72 hours and 5 and 7 days. Controls placed in methanol for similar times and temperatures.

Amine Groups by Deamination:

4. HNO_2,[5] 6 gm. $NaNO_2$, 5 cc. glacial acetic acid, water to make 40 cc. for 24 hours at 25 to 30 C.

Oxidant Actions:

5. Aqueous periodic acid (1%) for 10 and 30 minutes at 25 to 30 C. In addition to α-amino, β-hydroxy, and 1,2-glycol groupings, it attacks various phenols and other reducing groups.

6. $KMnO_4$ (0.5%) for 30 minutes at 25 to 30 C.

7. CrO_3 (5%) for 30 minutes at 25 to 30 C.

Removal of Ribonucleic Acid:

8. Digestion in 1:10,000 crystalline ribonuclease (Armour, lot No. 7095X) in pH 6 M/1 phosphate buffer for one hour at 37 C. Solvent controls in buffer without enzyme. Activity controls: sections of dog and rats pancreas and spinal cord similarly treated, as well as untreated sections, and followed by thionine.[5]

9. HNO_3 (0.2N) for 16 hours as 3 C. Similar activity and stain controls as Number 8, followed by thionine.[8]

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Removal of Glycogen:

10. Barley malt diastase (0.1%) in saline phosphate buffer of pH 6 for one hour at 37 C. Glycogen-laden liver section used as activity control. Stained by periodic acid-Schiff reaction.[9]

Aldehyde Blocking Procedures Interposed After 10-Minute Periodic Acid Oxidation to Confirm Aldehyde Nature of the Oxidation Product in Colloid:

11. Molar aniline chloride for 24 hours at 25 to 30 C. followed by Schiff reagent for 10 minutes, et cetera.[5]

12. Aqueous phenylhydrazine (5%) for 24 hours at 25 to 30 C. followed by Schiff reagent for 10 minutes, et cetera.[5]

Sulfhydryl Blockade with $HgCl_2$:

13. $HgCl_2$ (6%) for 16 hours at 20 to 25 C. followed by ferric ferricyanide reduction test. For procedure control, blocked and unblocked sections of young rabbit skin fixed in trichloracetic alcohol were used. The positive reactions in basal epidermis and root area of hair shafts were prevented by the $HgCl_2$.[6]

Other Direct Histochemical Reactions.—The other reactions are as follows:

14. Direct Schiff reaction for 10 minutes, 30 minutes, and 72 hours. The last is reactive to unsaturated compounds; the first two are reactive practically to native aldehydes only.

15. Peracetic acid-Schiff reaction for unsaturated compounds.[5]

16. Million reactions on alcohol, formalin, and Zenker's acetic-fixed tissues. It is said to be a reaction for tyrosine.[10]

17. Diazosafranine reaction, a coupling reaction for phenols with open ortho or para positions.[11]

18. Feulgen nucleal method for desoxyribonucleic acid.[5]

19. Feulgen-Voight plasmal reaction on frozen section for acetal phosphatides; Hayes variant.[12]

20. Oil red O, the supersaturated isopropanol method performed on frozen sections.[5]

21. Gomori's methenamine silver method for the presence of di-, polyhydroxy-, or NH_2-OH aryl compounds; Burtner-Lillie variant.[13]

22. Rinehart method for acid mucopolysaccharides[14] with and without pretreatment with hyaluronidase (Wyeth, lot No. 173A1, 1,500 TRU/mg.), 150 units per cubic centimeter in pH 5.5 sodium acetate-acetic acid buffer for 1 hour at 37 C. Control activity section of cock's comb used.

In Vitro Testing of Thyroxine with Ferric Ferricyanide.—In the testing of thyroxine, 10 mg. of thyroxine per 1 cc. distilled water and 1 cc. of ferric ferricyanide was used.

RESULTS

Effect of Fixation on Staining Reactions.—Thyroid colloid is stained by the periodic acid-Schiff procedure (red-purple), eosin, thionine (blue-basophilic but not metachromatic), ferric ferricyanide (blue-green), Millon's reagent (red-brown), and the Rinehart procedure (blue-green). The color produced with ferric ferri-

9. Lillie, R. D., and Greco, J.: Malt Diastase and Ptyalin in Place of Saliva in the Identification of Glycogen, Stain Technol. **22**:67-70, 1947. Lillie.[5]

10. Glick, D.: Techniques of Histo- and Cytochemistry: Manual of Morphological and Quantitative Micromethods for Inorganic, Organic, and Enzyme Constituents in Biological Materials, New York, Interscience Publishers, Inc., 1949.

11. Lillie, R. D.; Burtner, H. J. and Henson, J. G.: Diazo-Safranin for Staining Enterochromaffin, J. Histochem. & Cytochem. **1**:154-159, 1953.

12. Hayes, E. R.: A Rigorous Re-Definition of the Plasmal Reaction, Stain Technol. **24**:19-23, 1949.

13. Burtner, H., and Lillie, R. D.: A 5-Hour Variant of Gomori's Methenamine Silver Method for Argentaffin Cells, Stain Technol. **24**:225-227, 1949.

14. Rinehart, J. F., and Abul-Haj, S.: An Improved Method for Histologic Demonstration of Acid Mucopolysaccharides in Tissues, A. M. A. Arch. Path. **52**:189-194, 1951.

cyanide and the Millon's reagent is more brilliant after alcohol fixation. It might be noted, however, that variations in the intensity of the color produced by the ferric ferricyanide and Millon's procedures are more distinct when formalin fixation is utilized. A positive Rinehart reaction is obtained only after alcohol fixation. The other staining reactions do not appear to be dependent upon the fixative used.

Differences in Staining Reactions.—From Table 1 the results of various pretreatment procedures on the staining reactions are noted. Following acetylation or benzoylation only the periodic acid-Schiff reaction is inhibited. Methylation at 58 C. slightly impairs this reaction also, but only after 16 hours, and the impairment did

TABLE 1.—*Results of Blocking Procedures on Staining Reactions of Human Thyroid Colloid*

Reactions	Ferric Ferricyanide	Periodic Acid-Schiff	Thionine	Eosin
Color control	Blue-green	Red-purple	Blue	Red
Acetylation	No effect	Block	No effect	No effect
Benzoylation	No effect	Block	No effect	No effect
Methylation (0.05N HCl-CH_3OH)	No effect	Slight block (1 week, room temperature)	Block (4 hr., 58 C.) Block (16 hr., room temperature)	No effect
HNO_2	No effect	No effect	No effect	Slight reduction
Periodic acid (10 and 30 min.)	No effect	.....	No effect	Block
$KMnO_4$ (0.5%, 20 min.)	Block	No effect	No effect	No effect
Chromic acid (5%, 30 min.)	Block	No effect	Block	Block
Ribonuclease	.....	.....	No effect	.....
HNO_2	.....	.....	No effect	.....
Diastase	.....	No effect	.....	.....
Aldehyde blockade	No effect	Block (after periodic oxidation)	No effect	No effect
Saturated sublimate	No effect	.....	.....	.....
Direct Schiff (10 min. and 72 hr.)	Slight reaction (faint pink), same for both time intervals			
Peracetic acid-Schiff	Slight reaction (faint pink), similar to direct Schiff			
Millon	Positive (red-brown)			
Diazosafranine	Negative			
Feulgen nucleal	Negative			
Plasmal reaction	Negative			
Oil red O	Negative			
Methenamine silver	Negative			
Rinehart method	Positive (blue-green) only after alcohol fixation; unaffected by hyaluronidase pretreatment			

not increase up to three days. Thionine basophilia is completely abolished by methylation within 4 hours at 58 C., as well as in 16 hours at room temperature. The oxidants (periodic acid, $KMnO_4$, and chromic acid) inhibit eosin acidophilia but have no effect upon the periodic acid-Schiff reaction at the time intervals used. Basophilia is inhibited by chromic acid pretreatment only, and the ferric ferricyanide reduction is inhibited by both $KMnO_4$ and chromic acid but not by periodic acid.

Relationship of Various Staining Reactions to Functional Thyroid States.—Variation in the staining intensity of thyroid colloid is frequently observed with all of the staining procedures employed. This is found not only in the same section but also among individual cases considered to belong to a particular group of thyroid diseases. When compared with sections considered normal, these variations are also distinct. Generally speaking, there is uniformity in the variations noted with all of the staining procedures used, i. e., individual follicles that stain intensely with one

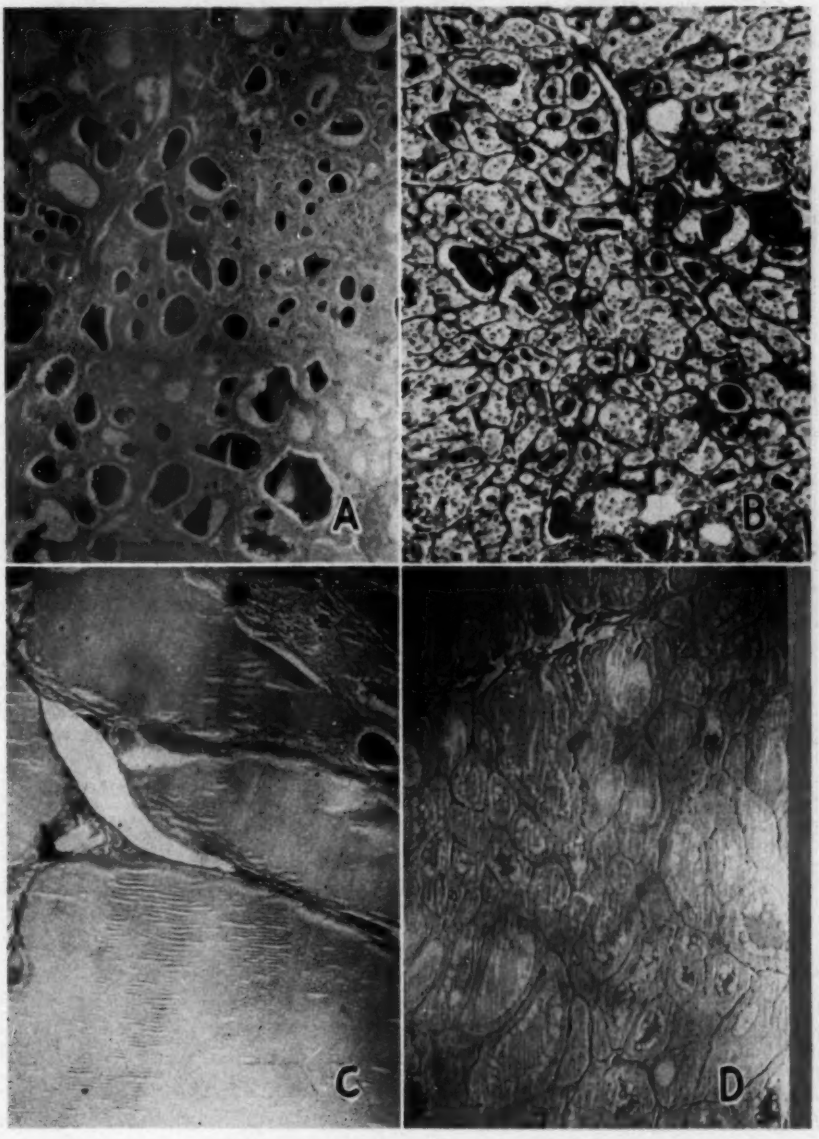


Fig. 1.—Human thyroid. *A*, variation in the intensity of thyroid colloid in an instance of struma lymphomatosa. Majority of follicles stain 4+. Ferric ferricyanide method; × 105. *B*, follicular colloid appears uniformly 4+ in an instance of struma lymphomatosa stained by the periodic acid-Schiff method; × 105. *C*, colloid goiter revealing the pale-staining large follicles, as compared to the intensely stained colloid of the small follicle. Ferric ferricyanide method; × 105. *D*, section of a hyperplastic, apparently hyperfunctioning, thyroid stained by the ferric ferricyanide method, demonstrating pale-staining follicular colloid. Compare with Figure 2*A*. × 105.

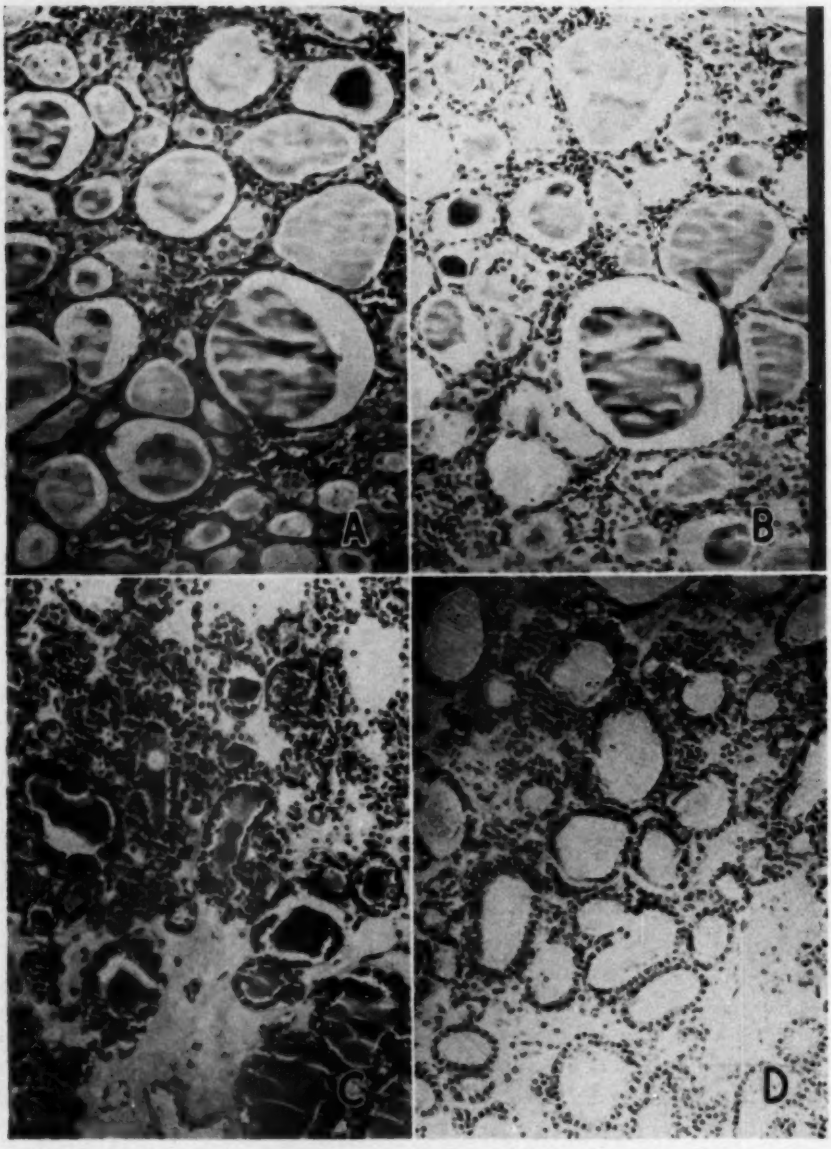


Fig. 2.—Human thyroid. *A*, section of thyroid stained with thionine revealing basophilic staining of colloid; × 125. *B*, similar section as in Figure 2*A* treated with crystalline ribonuclease prior to thionine staining. Note failure of removal of basophilia of colloid but clearing of cytoplasmic basophilia; × 125. *C*, control section of thyroid stained with thionine after being placed in methanol for 16 hours at room temperature. Colloid remains basophilic; × 125. *D*, similar section as in Figure 2*C*, although section was treated with the methylation procedure for 16 hours at room temperature. Note the removal of colloid basophilia; × 125.

stain are similarly affected by other stains. However, on occasion differences are noted, especially when comparing the ferric ferricyanide and the periodic acid-Schiff reactions. There is also a decided tendency for the colloid of hyperplastic, apparently hyperfunctioning glands (clinical history) to stain lightly, especially with the ferric ferricyanide method. On the other hand, the small follicles of struma lymphomatosa contain colloid that colors intensely with all stains. Large follicles in all thyroid states reveal a tendency to be lightly stained in comparison with smaller follicles in the same section. This variation is more consistently observed with the ferric ferricyanide reaction.

In the study of thyroid colloid from experimental animals, there appears to be more uniform results in comparing the staining reactions of the various induced

TABLE 2.—*Staining Intensity of Thyroid Colloid of Rats**

Rat No.	Condition	Eosin	Periodic Acid-Schiff	Thionine	Ferric Ferricyanide
1	Normal	Uniform 1+	Equal 4+, 1+	Uniform 1+	Uniform 1+
2	Normal	Uniform 1+	Equal 4+, 3+	Equal 2+, 1+	Uniform 1+
3	Normal	Uniform 1+	Equal 4+, 3+	Equal 2+, 1+	Uniform 1+
4	Normal	Majority 1+, (2+)	Equal 3+, 1+	Equal 2+, 1+	Uniform 1+
5	Normal	Majority 1+, (2+)	Equal 4+, 3+	Equal 2+, 1+	Majority 1+, (2+)
6	Hypophysectomized	Majority 2+, (1+)	Uniform 4+	Majority 3+, (1+)	Uniform 2+
7	Hypophysectomized	Majority 1+, (2+)	Equal 4+, 2+	Uniform 3+	Majority 2+, (1+)
8	Hypophysectomized	Equal 2+, 1+	Uniform 4+	Majority 2+, (3+)	Uniform 2+
9	Hypophysectomized	Uniform 2+	Uniform 4+	Uniform 2+	Majority 1+, (2+)
10	Hypophysectomized	Equal 2+, 1+	Equal 4+, 2+	Equal 2+, 1+	Uniform 2+
11	Thiourea-treated	Uniform 1+	Equal 4+, 2+	Equal 2+, 1+	Majority 1+, (2+)
12	Thiourea-treated	Equal 2+, 1+	Uniform 4+	Uniform 2+	Uniform 1+
13	Thiourea-treated	Equal 2+, 1+	Uniform 4+	Equal 2+, 1+	Uniform 1+
14	Hypophysectomized and thiourea-treated	Majority 2+, (1+)	Equal 4+, 3+	Uniform 2+	Uniform 2+
15	Hypophysectomized and thiourea-treated	Majority 2+, (1+)	Majority 4+, (3+)	Uniform 2+	Majority 2+, (3+)
16	Hypophysectomized and thiourea-treated	Equal 2+, 1+	Equal 4+, 3+	Uniform 2+	Uniform 2+
17	Hypophysectomized and thiourea-treated	Equal 2+, 1+	Majority 4+, (3+)	Equal 2+, 1+	Majority 2+, (1+)
18	Hypophysectomized and thiourea-treated	Uniform 1+	Uniform 4+	Equal 2+, 1+	Uniform 2+

* Equal indicates equal numbers of follicles; uniform indicates approximately all follicles; parentheses indicate occasional follicles.

thyroid states with the staining reactions of follicular colloid from the glands of untreated controls. This is most apparent with the ferric ferricyanide method (Table 2). It is noted that with this method colloid from hypophysectomized animals assumes a 2+ intensity, which tends to be uniform among the follicles in a given section. Those animals receiving thiourea demonstrate an intensity of 1+, similar to that observed in the normal animals. The follicular colloid of those receiving thiourea after hypophysectomy resembles that of hypophysectomized animals. The other procedures employed revealed greater variability within the same thyroid section and less distinction between the various thyroid states than the ferric ferricyanide reaction.

In Vitro Reaction.—The mixture of 1 cc. of ferric ferricyanide and 10 mg. of thyroxine in 1 cc. of distilled water at room temperature results in the development of a blue-green color (positive) in 10 minutes and a pale yellow-green filtrate with a blue precipitate in one hour.

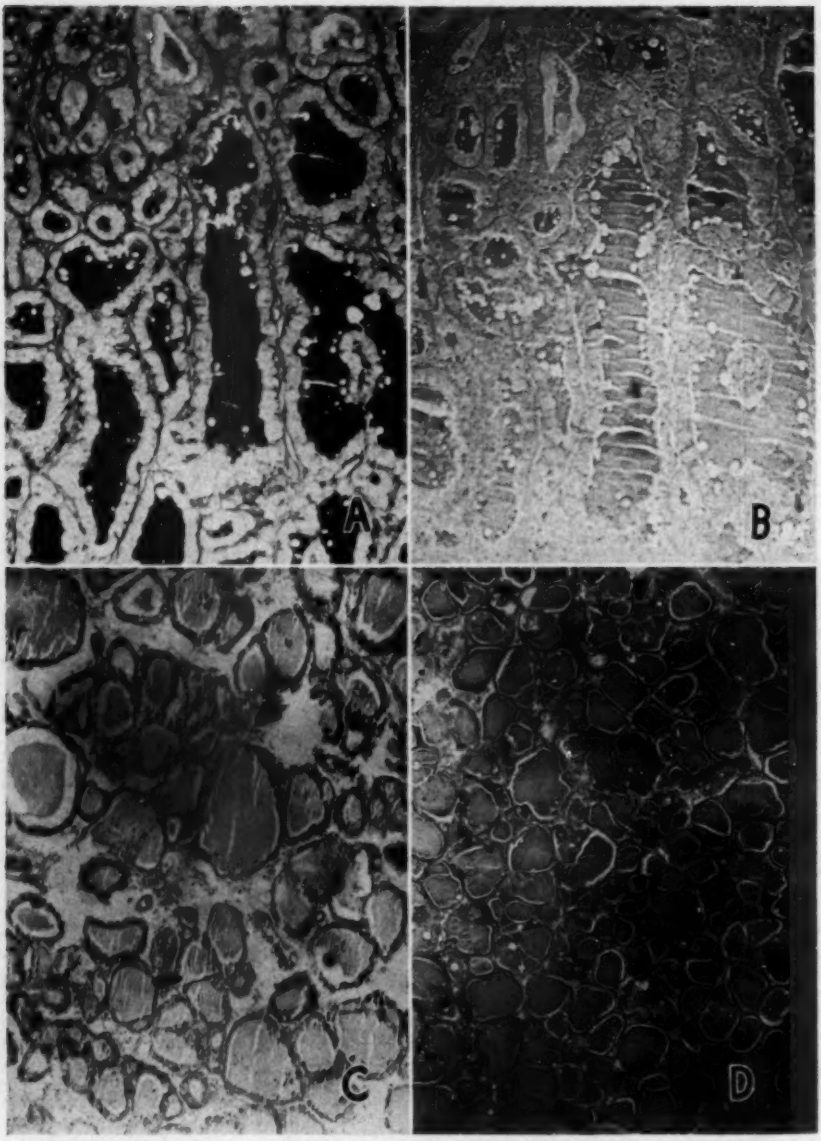


Fig. 3.—*A*, section of a hyperplastic, apparently hyperfunctioning human thyroid stained by the periodic acid-Schiff method. The colloid assumes a 4+ intensity. Compare with Figure 1*D*; × 125. *B*, similar section to that in Figure 3*A* stained by the direct Schiff method. The colloid stains like the background; × 125. *C*, normal rat thyroid stained by the ferric ferricyanide procedure; × 105. *D*, hypophysectomized rat thyroid stained by the ferric ferricyanide method. Note the more intense staining of the colloid than in Figure 3*C*; × 105.

COMMENT

Although Dempsey [1] believed that the basophilia of thyroid colloid was the result of its ribonucleoprotein content, we have had no success in the destruction of this basophilia by either enzymatic (crystalline ribonuclease) or acid (HNO_3) methods. The prompt inhibition of this reaction by mild methylation indicates that basophilia may more likely be due to the presence of COOH (or $=OSO_3H$) groups in thyroid colloid.

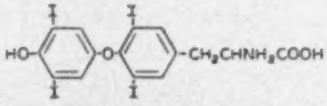
The investigation of the positive periodic acid-Schiff reaction of colloid revealed that such a reaction is not due to glycogen, since it is unaffected by diastase digestion. Further, the periodic acid-Schiff reaction is inhibited by acetylation and benzoylation, indicating the presence of either hydroxyl or amine groups. Although thyroid colloid gives a faint direct Schiff reaction (faint pink), the intensity obtained is little different after 10 minutes or 72 hours and is similar to that noted with the peracetic acid-Schiff procedure. Such results raise doubt as to the actual significance of the direct Schiff reaction of colloid and eliminate the presence of ethylenic linkages.

The ferric ferricyanide reduction test and its use in histochemistry has recently been investigated by Lillie and Burtner.[6] They have noted that ascorbic, oxalic, and uric acids; phenols, including tyrosine, indols, pyrrols, arylamines, hydrazines, thiols, and inorganic sulfides, and peroxide promptly reduce ferricyanide mixtures to Prussian blue. A positive reaction with thyroid colloid in tissue sections and in vitro with thyroxine has not been previously reported; although, because of its reaction with tyrosine, it would seem likely that it would react with di-iodotyrosine and thyroxine. That the positive reaction with colloid is not due to sulfhydryl groups is evident by the failure of blockade by saturated sublimate. In addition, if it were due to oxalic or uric acid one would expect to note these substances in the crystalline form, which is not apparent in any of the frozen sections studied. Since no particular precautions to preserve ascorbic acid were used in this study, it seems unlikely that the reaction is of this nature. The quantity of other reacting substances mentioned appears unlikely to be of significant magnitude to account for the reaction. Further, one may eliminate the possibility of the reaction being due to tyrosine per se, since there is lack of diazo coupling (diazosafranine reaction) with thyroid colloid; yet coupling with thyrosine should occur owing to the open ortho position in this substance. This indicates that ferric ferricyanide probably reacts in the instance of colloid with the phenolic portion of a substance that has ortho and para positions occupied, since diazo coupling occurs only with substances possessing phenolic structure having unoccupied para or ortho positions.

The nature of the acidophilia of thyroid colloid at present is not apparent from the reactions studied. However, the reaction is slightly impaired by the HNO_2 deaminating procedure and is completely inhibited by chromic acid oxidation.

The observation that thyroid colloid stains more brilliantly after alcoholic fixation suggests that the intensity of the reactions is at least in part dependent upon the presence of a water-soluble, alcohol-insoluble substance. It is interesting to note that contrary to current concepts the Millon reaction may be observed after the use of aqueous fixation, although the reaction is not as intense as that observed after alcohol fixation. This, as well as the negative diazosafranine reaction, suggests that the Millon reaction of thyroid colloid may be due to the presence of a tyrosine derivative, rather than of tyrosine per se.

Analyzing the chemical structure of thyroxine, which is alcohol insoluble but water soluble,



one may see that the basophilia of thyroid colloid may be in part due to the reactive COOH group present. The periodic acid-Schiff reaction, however, must be theoretically explained as being due to the presence of some other substance in thyroid colloid, since susceptible reactive groups in the thyroxine molecule are not present. The results of this study suggest that a mucopolysaccharide present in thyroid colloid accounts for the reaction. Gersh's [15] studies have indicated that the positive periodic acid-Schiff reaction is due to the presence of polysaccharide and that at least part of the visualized polysaccharide represents hyaluronic acid. This hypothesis was based on the positive periodic acid-Schiff reaction of thyroid colloid and the observation that the thyroid gland is very rich in hyaluronidase and that the enzyme alters the viscosity of the colloid. In addition, he [16] has indicated that a glycoprotein resembling thyroglobulin is also responsible for the periodic acid-Schiff reaction. Further support to this hypothesis is added by our observation that colloid stains positively with the Rinehart method only after alcoholic fixation. Grishman [17] has insisted on the use of fixatives of high alcoholic content for the demonstration of hyaluronic acid "because of the high solubility of hyaluronic acid in water." Yet, the failure to inhibit the periodic acid-Schiff and Rinehart reactions with hyaluronidase (control sections show inhibition) fails to support the hyaluronic acid nature of the polysaccharide of thyroid colloid. The positive ferric ferricyanide reaction may be explained as being due at least in part to the phenolic structure of thyroxine, as mentioned above. Further support is added to this hypothesis by the positive in vitro reaction obtained with thyroxine. The slight reduction in acidophilia following deamination may be due to the NH_2 group present in thyroxine or to those in other protein residues. It becomes apparent that thyroid colloid is of complicated structure. Salter [18] has summarized this by the statement, "the framework of thyroglobulin may be a true biochemical entity, albeit a complicated one."

The correlation of the staining of thyroid colloid with function, for the most part, has been disappointing, especially in the human material. Yet, this is not too surprising, since certain biochemical and physiologic features of thyroid colloid as yet remain unanswered. However, there is a definite tendency for the thyroid colloid of apparently hyperfunctioning glands to stain lightly, whereas that of supposed hypofunctioning states stains intensely. This is in accord with the observations that in hyperactive states there is little storage of hormone. The proposed explanation

15. Gersh, I.: Polysaccharide Complex in Individual Follicles of the Thyroid Gland of the Rat, Federation Proc. **6**:392, 1947.

16. Gersh, I.: Glycoproteins in the Thyroid Glands of Rats, J. Endocrinol. **6**:282-287, 1950.

17. Grishman, E.: Histochemical Study of Mucopolysaccharides in Various Normal Tissues, Bull. Internat. A. M. Mus. **28**:104-118, 1948.

18. Salter, W. T.: The Endocrine Function of Iodine, Cambridge, Mass., Harvard University Press, 1940.

of the various staining reactions observed leads one to hypothesize that perhaps not only the thyroxine portion of thyroid colloid is reabsorbed during periods of hyperactivity but that other portions are similarly affected.

SUMMARY

The nature of the staining reactions of thyroid colloid has been investigated. As the result of the various histochemical procedures employed, it is felt that thionine basophilia may best be explained as the result of COOH (or OSO_3H) groups, rather than due to the presence of ribonucleoprotein. The periodic acid-Schiff reaction of thyroid colloid is dependent upon the presence of either 1,2-glycols or $OH\text{-}NH_2$ linkages, which indicates, in the presence of a positive Rinehart reaction, a mucopolysaccharide. Although there is some evidence that this may represent hyaluronic acid, there is a discrepancy noted, since these reactions are not inhibited with hyaluronidase. The ferric ferricyanide reduction test, which reacts with thyroid colloid in tissue sections, also reveals a positive reaction with thyroxine in vitro. The possibility of this reaction in colloid being due to its thyroxine content is discussed.

Generally there is uniformity of variation in the color intensity of thyroid colloid by the different staining procedures. The divergent results obtained by various pretreatments, as well as occasional staining differences, indicate that the nature of the staining reactions are different. The possibility that not only the active principle of thyroid colloid is reabsorbed is also proposed to account for those instances in which there is uniformity among the various staining procedures.

It has been found with moderate consistency that the colloid of hyperplastic and apparently hyperfunctioning human thyroids stains lightly, whereas the colloid in those thyroids recognized as hypofunctional states stains intensely. There is agreement noted with the thyroids studied experimentally.

HYPERTENSION IN BILATERALLY NEPHRECTOMIZED DOGS IN ABSENCE OF EXOGENOUS SODIUM EXCESS

Maintenance of Fluid Balance During Peritoneal Irrigation

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SINCE the demonstration that a significant and sustained hypertension develops frequently in bilaterally nephrectomized dogs maintained by in vivo dialysis,[1] several workers [2] have presented evidence indicating a relationship between the retention of fluid and salt (sodium) in these animals and the development of the hypertension. In earlier series of dogs maintained by an artificial kidney or peritoneal irrigation [3] a gain in the dialyzing fluid occurred which was associated with a gain in weight in some animals. Moreover, it has been demonstrated that rats developing hypertension under various conditions,[4] including bilateral nephrec-

Supported by a grant from the National Institutes of Health, Cardiovascular Study Section.

From the Department of Pathology, Southwestern Medical School of the University of Texas.

1. Grollman, A.; Muirhead, E. E., and Vanatta, J.: Role of the Kidney in Pathogenesis of Hypertension as Determined by a Study of the Effects of Bilateral Nephrectomy and Other Experimental Procedures on the Blood Pressure of the Dog, Am. J. Physiol. **157**:21, 1949.

2. (*a*) Leonards, J. R., and Heisler, C. R.: Artificial Kidney: Maintenance of Life in Bilaterally Nephrectomized Dogs and Its Relation to Malignant Hypertension, Am. J. Physiol. **167**:553, 1951; (*b*) Blood Pressure of Bilaterally Nephrectomized Dogs, Fed. Proc. **11**:247, 1952. (*c*) Houck, C. R.: Experience with Intermittent Peritoneal Dialysis for Keeping Alive Dogs Without Kidneys, ibid. **11**:74, 1952; (*d*) Effect of State of Hydration on Hypertension in Dogs Living a Month or Longer Without Kidneys, ibid. **12**:70, 1953. (*e*) Orbison, J. L.; Christian, C. L., and Peters, E.: Studies on Experimental Hypertension and Cardiovascular Disease: A Method for the Rapid Production of Malignant Hypertension in Bilaterally Nephrectomized Dogs, A. M. A. Arch. Path. **54**:185, 1952. (*f*) Kolff, W. J.: Treatment of Uremia, read before the Clinical Session, American Medical Association, Denver, Dec. 4, 1952. (*g*) Masson, G. M. C.; Plahl, G.; Corcoran, A. C., and Page, I. H.: Accelerated Hypertensive Vascular Disease from Saline and Renin in Nephrectomized Dogs, A. M. A. Arch. Path. **55**:97, 1953.

3. (*a*) Muirhead, E. E.; Grollman, A., and Vanatta, J.: Hypertensive Cardiovascular Disease ("Malignant Hypertension"): Changes in Canine Tissues Induced by Various Manipulations of the Kidney, with Special Reference to Vascular and Myocardial Lesions, Arch. Path. **50**:137, 1950. (*b*) Grollman, A.; Turner, L. B., and McLean, J. A.: Intermittent Peritoneal Lavage in Nephrectomized Dogs and Its Application to the Human Being, A. M. A. Arch. Int. Med. **87**:379, 1951. (*c*) Muirhead, E. E.; Stirman, J. A.; Jones, F.; Lesch, W.; Burns, M., and Fogelman, M. J.: Cardiovascular Lesions Following Bilateral Nephrectomy of Dog: Role of Hypertension and Other Factors on Pathogenesis, ibid. **91**:250, 1953. (*d*) Footnote 1.

4. Braun-Menéndez, E.: Blood Volume and Extracellular Fluid Volume in Experimental Hypertension, in Hypertension, edited by E. T. Bell, Minneapolis, University of Minnesota Press, 1951, p. 98.

tomy, reveal an expansion of the "extracellular fluid space" and edema. Thus, it has been considered that the hypertension of bilateral nephrectomy represents an expression of the sodium retention.

In the present discussion we wish to present data supporting the view that the hypertension following renal ablation of the dog may occur in two different preparations in the absence of exogenous sodium excess and while the animals are maintained in fluid balance in one of these preparations.

MATERIALS AND METHODS

Adult mongrel dogs of either sex weighing between 9 and 19 kg. were used. Two experimental groups were studied. The first group consisted of dogs subjected to bilateral nephrectomy and given no water or food by any route. The second group consisted of animals maintained in fluid balance during the peritoneal dialysis after the second nephrectomy for periods varying between 7 and 11 days. The input and output volume and sodium content of the peritoneal (irrigating) fluid were measured. In the two groups only those animals revealing negligible or no vomiting and no diarrhea during the experimental period were included in the series.

The mean arterial pressure was taken daily by means of direct femoral artery puncture and a mercury manometer. Peritoneal irrigation was conducted intermittently by introducing and removing 2,000 to 3,000 cc. of fluid into and out of the peritoneal cavity each 24 hours. The fluid

TABLE 1.—*Changes in Mean Arterial Pressure in Five of Eleven Dogs in Group 1*

Dog	Control Blood Pressure, Mm. Hg	Hypertensive Blood Pressure, Mm. Hg	Change in Blood Pressure, Mm. Hg
1	120	180	+60
2	117	145	+28
3	117	150	+33
4	117	160	+43
5	120	140	+20

remained in the peritoneal cavity for 6 to 14 hours. The fluid was introduced by gravity and removed by means of a needle and a rubber tube without suction. The composition of the fluid expressed as milliequivalent per liter was as follows: sodium 141, potassium 2.6, calcium 2.6, magnesium 0.6, chloride 104.8, bicarbonate 41. In addition the fluid contained 20 or 30 gm. of dextrose and 100 mg. of terramycin (Chas. Pfizer & Company, Inc.) per liter.

The sodium concentration of the fluid and serum was determined by the gravimetric method of Butler and Tuthill.[5] The potassium concentration was determined by the methods of Breh and Gaebler as modified by Cumings and by the method of Jacobs and Hoffman.[6] The blood volume was estimated by the T-1824 hematocrit method as discussed by Mayerson and coworkers.[7] The plasma urea was determined by the method of Blair and Taylor.[8] The nitrogen concentration of the outflow peritoneal fluid was determined by micor-Kjeldahl digestion and direct Nesslerization.[9] The plasma protein concentration in the plasma and peritoneal fluid was determined by the method of Greenberg.[10]

5. Butler, A. M., and Tuthill, E.: An Application of the Uranyl Zinc Acetate Method for Determination of Sodium in Biological Material, J. Biol. Chem. **93**:171, 1931.

6. Breh, F., and Gaebler, O. H.: The Determination of Potassium in Blood Serum, J. Biol. Chem. **87**:81, 1930. Cumings, J. N.: The Estimation of Potassium by Micro-Method, J. Clin. Path. **1**:173, 1948. Jacobs, H. R. D., and Hoffman, W. S.: New Colorimetric Method for Estimation of Potassium, J. Biol. Chem. **93**:685, 1931.

7. Mayerson, H. S.; Lyons, C.; Parsons, W.; Nieset, R. T., and Trautman, W. V.: Comparison of Results of Measurement of Red Blood Cell Volume by Direct and Indirect Technics, Am. J. Physiol. **155**:232, 1948.

8. Taylor, W. F., and Blair, W. M.: Improved Method for Determination of Blood Urea Nitrogen by Direct Nesslerization, J. Lab. & Clin. Med. **17**:1256, 1932.

9. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**:81, 1919.

10. Greenberg, D. M.: The Colorimetric Determination of the Serum Proteins, J. Biol. Chem. **82**:545, 1929.

The animals were weighed each day after the withdrawal of the peritoneal fluid. The diet consisted of protein (0.3 to 3.0 gm. per kilogram of body weight), peanut or coconut oil, dextrose and vitamin supplements as previously described.[3c] The caloric intake was derived approximately equally from fat and carbohydrate and varied between 47 and 140 Cal. per kilogram of body weight per day (average 57 Cal./kilogram per day). The dietary formula was given in several different volumes (Table 2). Water for the insensible needs was derived from this volume and the water of metabolism. The estimated requirements were not necessarily attained in an attempt to avert "water intoxication." The operative procedure was previously described.[3c]

RESULTS

Hypertension was considered significant when the mean arterial blood pressure was elevated to +25 mm. Hg or more above the control level. The control level consisted of the pressures taken during the week prior to the second nephrectomy.

Group 1: The 11 dogs in this group were given no food or water after the second nephrectomy. They survived for four to five days. Four of the 11 animals (36%) displayed a mean arterial pressure of +28 to +60 mm. Hg on the third

TABLE 2.—*Features During Fluid Balance*

Dog	Weight, Kg.	No. Days	Peritoneal Fluid Balance, Cc. In	Peritoneal Fluid Balance, Cc. Out	Peritoneal Sodium Balance, mEq. In	Peritoneal Sodium Balance, mEq. Out	Serum NA, MEq./Liter*	Blood Vol. Change, %	Blood Pressure Change, Mm. Hg	Dietary Formula, Cc./Day	Plasma Urea, Mg./100 Cc.*
1.....	9.1	7	19,000	18,600	2,680	2,905	141 136	+5	+70	80	74 320
2.....	11.0	8	22,000	21,200	3,100	3,223	....	—14	+35	60	110 227
3.....	11.5	11	32,000	32,200	4,510	4,559	148 139	—15	+30	90	70 451
4.....	11.0	10	27,000	25,340	3,810	3,849	151 167	—16	+45	85	80 470
5.....	10.0	8	17,000	17,000	2,400	2,421	140 140	0	+35	470	70 590
6.....	11.5	10	28,000	27,500	3,950	3,751	151 135	—25	+35	180	35 235
7.....	10.7	7	13,000	12,600	.....	.....	....	+17	+50	100	.. 700
Average..	12.0	8.7	22,570	22,000	3,408	3,451	148 143	+8	+43	152	73 427

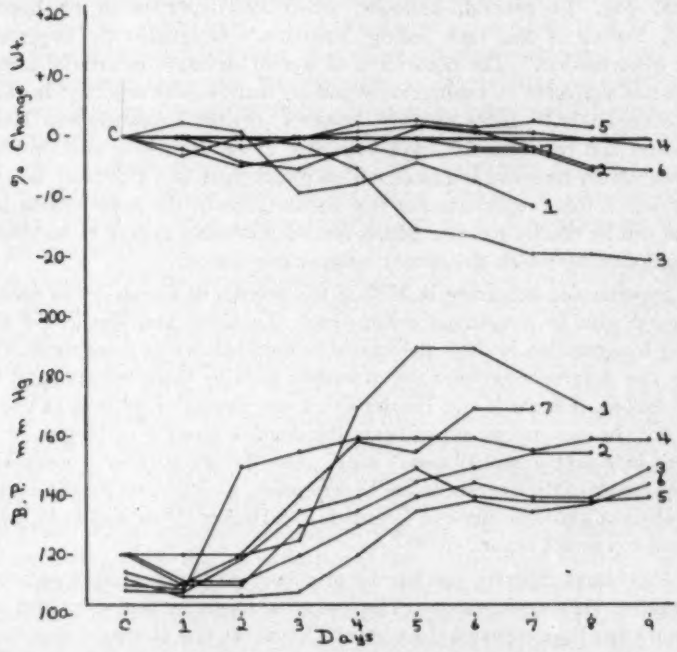
* First value before second nephrectomy, second value at end of balance period.

or fourth day (Table 1). An additional dog revealed a +20 mm. Hg pressure on the second day. This hypertension was short-lived, being followed within 24 hours by hypotension and circulatory collapse.

Group 2: There were 20 dogs in Group 2, of which 7 (35%) developed a sustained hypertension amounting to +30 to +70 mm. Hg above the control pressure. The progression of the hypertension during the balance period is displayed in the Chart. There was a lag of four to six days before the arterial pressure reached +25 mm. Hg or more, although during this period slight elevations occurred. The body weight was unchanged or reduced in five of the seven dogs. The other two dogs revealed a slight (2 to 3%) elevation in weight.

In Table 2 the main features of the balance studies are shown. The volume output of fluid either equaled or almost equaled the input. At the same time the total quantity of sodium obtained (daily volume and concentration values) slightly exceeded the input in five dogs and was equal to it in one. The serum sodium concentration was depressed in four of five animals tested. While the hypertension developed, the blood volume was either slightly decreased or increased, and azotemia became prominent.

Table 3 contains data concerning the protein and caloric intake and the nitrogen loss via the irrigating fluid. The animals were placed on a low but definite protein intake. The caloric intake was adequate to prevent starvation, but nevertheless a



The daily mean arterial blood pressure and percentile change in body weight are depicted. The designation "C" is for the mean control values taken for one week before the second nephrectomy. The lower figure relates the blood pressure as taken daily. The control blood pressure varied between 115 and 120 mm. Hg. The elevation in pressure was usually gradual up to five days; between five and seven days the maximal values were attained (140 to 190 mm. Hg). Five of the seven dogs lost weight. A slight increment in weight occurred transiently in three dogs (2 to 3%). An unknown degree of weight loss resulted from the emptying of the intestinal contents and the insensible water loss.

TABLE 3.—*Protein and Caloric Intake and Nitrogen Output in Peritoneal Fluid*

Dog	Weight, Kg.	No. Days	Average Protein Intake, Gm./Kg./ Day	Average Caloric Intake, Cal./Kg./ Day	Average Nitrogen Intake, Gm./Kg./ Day	Average Nitrogen in Fluid, Gm./Kg./ Day	Emeses	Diarrhea
1.....	9.1	7	1.2	75	0.19	0.52	0	0
2.....	11.0	8	0.5	75	0.08	1.35	0	0
3.....	11.5	11	0.4	53	0.064	1.2	2	0
4.....	11.0	10	0.37	47	0.06	0.73	2	0
5.....	19.0	8	2.0	40	0.48	0.57	0	0
6.....	11.5	10	1.5	93	0.24	1.4	0	0
7.....	10.7	7	1.1	140	0.18	—	0	0
Average.....	12.0	8.7	1.15	57	0.18	0.96	..	..

prominent negative nitrogen balance developed. The loss of nitrogen became more pronounced during the second half of the balance period, while the hypertension and azotemia reached their maxima.

COMMENT

Earlier experiments dealing with bilateral nephrectomy of the dog by Winternitz and co-workers [11] and Harrison and co-workers [12] yielded hypertension in an occasional dog. In general, however, either no hypertension or hypotension occurred. Struck by this rare finding, Harrison [12] designated the hypertension a "uremic hypertension." The occurrence of a mild elevation in arterial pressure in such animals subjected to nephrectomy and no dialysis was observed in this institution subsequently.[13] More recently, however, neither Leonards and Heisler [2a,b] nor Orbison and co-workers [2c] have been able to confirm these observations. The experience herein recorded indicates not only that there is a relatively low yield of hypertension in this preparation but that the duration of the hypertension is short-lived and can be readily missed. Moreover, such animals appear to be sensitive to vomiting, following which circulatory collapse is common.

The hypertension occurring in 35% of the animals in Group 2 was definite and unassociated with an exogenous sodium load. Leonards and Heisler [2a,b] failed to encounter hypertension in dogs maintained in fluid balance as these were. There is, however, one difference between the procedure used by these workers and the one herein recorded. Leonards and Heisler's diet was devoid of protein, as our earlier diets were.[1] In the present experiments the dogs received 4 to 11 gm. of protein (complete in essential amino acids) daily. The dietary protein is considered an important factor in the genesis of the hypertension, as indicated by the comparison of these observations with those of Leonards and Heisler,[2a,b] as well as by additional observations reported separately.[14]

The dogs herein reported developed a prominent negative nitrogen balance while being irrigated via the peritoneum. The loss of nitrogen, as well as plasma protein, by this route has been reported elsewhere.[15] Leonards and Heisler's observation,[2a,b] as well as our earlier and subsequent observations,[16] indicate that dogs depleted in this manner, when allowed to gain fluid and sodium, develop a hypertension. Also, dogs may develop hypertension following nephrectomy in the absence of in vivo dialysis as shown on this and former occasions.[17] Moreover, a transient hyper-

11. Winternitz, M. C.; Mylon, E.; Waters, L. L., and Katzenstein, R.: Studies on the Relation of the Kidney to Cardiovascular Disease, Yale J. Biol. & Med. **12**:623, 1940.

12. Harrison, T. R.; Mason, M. F.; Resnik, H., and Rainey, J.: Changes in Blood Pressure in Relation to Experimental Renal Insufficiency, Tr. A. Am. Physicians **51**:280, 1936.

13. Muirhead, E. E.; Vanatta, J., and Grollman, A.: Hypertensive Cardiovascular Disease: Experimental Study of Tissue Changes in Bilaterally Nephrectomized Dogs, Arch. Path. **48**:234, 1949.

14. Muirhead, E. E.; Fogelman, M. E.; Jones, F., and Graham, P.: Hypertension in Bilaterally Nephrectomized Dogs in the Absence of Exogenous Sodium Excess and Without ECF Expansion, Fed. Proc. **12**:397, 1953. Muirhead, E. E.; Jones, F., and Graham, P.: Hypertension Following Bilateral Nephrectomy of the Dog: The Role of Dietary Protein on Its Pathogenesis with Emphasis on Its Development in the Absence of "Extracellular Fluid" Expansion, Circulation Res. to be published.

15. Muirhead, E. E.; Jones, F.; Stirman, J. A., and Lesch, W.: Experimental Uremia: Excessive Quantities of Plasma Protein and Nitrogen Removed in the Outflow Fluid During Peritoneal Irrigation of Bilaterally Nephrectomized Dogs, Surg., Gynec. & Obst., to be published.

16. Footnote 1. Footnote 15.

17. Footnote 11. Footnote 12. Footnote 13.

tension may occur in dogs given no food or water and consequently having no access to exogenous sodium.

From reports in the literature and the additional observations recorded herein, it is evident that hypertension may not be manifested in certain experimental preparations of bilateral nephrectomy. Hypertension may be either rare or transient following bilateral nephrectomy alone; it is either nonexistent or almost so following bilateral nephrectomy alone plus salt depletion, as by vomiting by itself or subsequent to the ingestion of water or food; it was not encountered following bilateral nephrectomy and dialysis attended by no protein in the diet [18] and salt balance,[19] although some of these animals vomited and may have been depleted of sodium. Thus, the advanced "uremic state" or potassium intoxication, sodium depletion, and marked protein depletion appear to be deterrants to the expression of hypertension after renal ablation.

Hypertension following nephrectomy has been most consistent with four preparations: (*a*) with dialysis, no protein intake and salt (sodium) loading [2a,b]; (*b*) with dialysis, a mild to moderate protein intake [2a,b] (0.3 to 1 gm. per kilogram of body weight per day) and salt loading [18]; (*c*) with dialysis, a moderate to marked protein intake (2 to 3.5 gm. per kilogram of body weight per day) and salt balance,[14] and (*d*) with no dialysis and a sudden hypervolemia. The last statement (*d*) is based mainly on unpublished data. However, this factor may well have played a part in the abrupt and pronounced hypertension encountered by Orbison and co-workers.[2e] Their nephrectomized animals received a balanced salt solution at the rate of 100 ml. per kilogram of body weight per day. The observations of Warren, Merril, and Stead, indicating an influence of the extracellular fluid on the plasma volume, suggest a hypervolemia under these conditions. However, the surmised hypervolemia is not considered the only factor in the hypertension, as it likely was operating within and potentiating the increased peripheral vascular resistance which has been indicated following the nephrectomies.[21] The present data support a fifth but possibly less consistent preparation, that of bilateral nephrectomy plus salt balance and a mild to moderate protein intake wherein at least 35% of the animals became significantly hypertensive.

Thus, hypertension appears to be a fundamental disturbance associated with the removal of the kidneys. In the absence of food and electrolyte intake and dialysis the pathogenesis of the hypertension must be related to endogenous factors. One endogenous factor unique to these experiments is the absence of renal function. Altered renal function in the sense of a deficiency state as the main factor in the pathogenesis of hypertension has been championed by Grollman for years.[22] That other factors are at play in the pathogenesis of the hypertension following renal ablation is suggested by the present and other observations.[23]

18. According to our observations such animals are depleted of protein.

19. Footnote 2 *a*, *b*, and *c*.

20. Warren, J. V.; Merrill, A. J., and Stead, E. A., Jr.: The Role of the Extracellular Fluid in the Maintenance of a Normal Plasma Volume, J. Clin. Invest. **22**:635, 1943.

21. Grollman, A.: Hemodynamics of Bilaterally Nephrectomized Dog Subjected to Intermittent Peritoneal Lavage, Am. J. Physiol. **165**:167, 1951.

22. Grollman, A.: Experimental Studies on Hypertension, in Hypertension: Symposium held at University of Minnesota on September 18, 19, and 20, 1950, in Honor of Elexious T. Bell, Benjamin J. Clawson, and George E. Fahr, edited by E. T. Bell, Minneapolis, University of Minnesota Press, 1951, p. 22.

23. Footnote 2. Footnote 14. Footnote 15.

The hypertension following bilateral nephrectomy of the dog appears to be influenced by at least four factors: (*a*) a sudden increment in blood volume; (*b*) salt (sodium) loading; (*c*) the intake (dietary) of protein, and (*d*) endogenous factors unrelated to diet, sodium intake, or blood volume.[24] In view of the potentiating influence of dietary protein on the hypertension, the endogenous factors may be related to the endogenous breakdown of body protein. The elucidation of the pathogenesis of this type of experimental hypertension appears related to the relationship of the sodium intake and/or sodium metabolism, the protein intake and its metabolism, and the absence of renal function. Observations are in progress concerning this relationship.

CONCLUSIONS

Dogs subjected to bilateral nephrectomy and given no water or food occasionally develop a significant but transient hypertension.

Dogs maintained in fluid and sodium balance after bilateral nephrectomy while being dialyzed via the peritoneum may develop hypertension under certain conditions. The two main requisites for the development of hypertension under these conditions appear to be (*a*) the absence of vomiting and diarrhea as sources of salt depletion and (*b*) the addition of a complete protein to the diet.

The present and other observations indicate that three factors can potentiate the hypertension of nephrectomy: (*a*) sodium loading, (*b*) hypervolemia, and (*c*) dietary protein. The first 2 factors were controlled in the present experiments. The manner in which dietary protein potentiates the hypertension requires further observations.

The influence of dietary protein on the hypertension suggests the possibility that the endogenous breakdown of body protein may also be involved in the pathogenesis of the hypertension.

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24. Muirhead, E. E.; Jones, F.; Stirman, J. A., and Lesch, W.: Hemolytic Anemia of the Dog Following Bilateral Nephrectomy, Am. J. Physiol. **173**:371, 1953; Anemia of Renal Insufficiency (Uremia) as Observed in Dogs Following Bilateral Nephrectomy and Maintenance by Peritoneal Irrigation: Evidence for a Hemolytic Mechanism, Fed. Proc. **11**:110, 1952.

HEMATOXYLIN BODIES ASSOCIATED WITH ALLERGIC ANGIITIS IN ABSENCE OF LUPUS ERYTHEMATOSUS

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SINCE 1932, when Gross [1] first called attention to peculiar structures which he designated "hematoxylin bodies," such structures have been found characteristically in acute lupus erythematosus. Klemperer and co-workers [2] have stated that agglomerations of these bodies in large masses occur only in this disease. In the report to be presented of a case of asthma in which the necropsy revealed a fulminating allergic angiitis and interstitial myocarditis, aggregates of hemotoxylin bodies were widely disseminated in many organs and tissues. This appears to be the first instance in which hematoxylin bodies have been encountered in the absence of systematized acute lupus erythematosus.

REPORT OF A CASE

Clinical Summary.—A 23-year-old Puerto Rican man was admitted to the Veterans Administration Hospital in May, 1952. His illness began with severe unremitting attacks of bronchial asthma five months after his induction into the Army in January, 1951. He was hospitalized from June, 1951, to January, 1952, and was treated with a variety of medications, including penicillin, aureomycin, and chloramphenicol (Chloromycetin). On discharge from the Army in January, 1952, he was only mildly symptomatic but had lost about 25 lb. (11.3 kg.).

From January until May, 1952, mild asthmatic attacks relieved by epinephrine (adrenalin) continued. Three weeks before admission he had a severe asthmatic attack accompanied by fever, malaise, and nonproductive cough. Penicillin and aureomycin were administered, with symptomatic improvement, although a low-grade fever and malaise persisted. Two weeks before admission he developed orthopnea, anorexia, and tenderness in the right upper quadrant.

There was no past history of allergy. One brother was reported to have had mild asthmatic attacks. The patient came to New York City from Puerto Rico when he was 10 years of age. There had been no unusual childhood diseases.

Physical examination revealed a thin apprehensive man appearing chronically ill. The blood pressure was 104 mm. Hg systolic and 80 mm. Hg diastolic. The head, eyes, ears, and nose were negative. A sinus tachycardia was interrupted by frequent premature contractions; aortic second sound equaled pulmonic second sound, and there were no murmurs or evidence of cardiac enlargement. The liver was tender and enlarged 6 cm. below the right costal margin. The skin showed mild pallor but no rash or jaundice. No abnormal neurological findings could be elicited.

The initial laboratory findings were as follows: Urinalysis showed specific gravity 1.016, a trace of albumin, and two to three hyaline casts and one to three granular casts per high-power

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1. Gross, L.: The Heart in Atypical Verrucous Endocarditis (Libman-Sacks), in Libman Anniversary Volumes, New York, International Universities Press, 1932, Vol. 2, p. 527.

2. Klemperer, P.; Gueft, B.; S. L.; Leuchtenberger, C., and Pollister, A. W.: Cytochemical Changes of Acute Lupus Erythematosus, Arch. Path. **49**:503, 1950.

filed. Blood studies gave the following values: red blood cells 3,600,000; hemoglobin 12.3 gm. per 100 cc.; white blood cells 11,000, with 78% neutrophiles, 9% lymphocytes, 3% monocytes, and 10% eosinophiles. Sedimentation rate was 30 mm. in one hour. The serum bilirubin was 0.4 mg. per 100 cc., the thymol turbidity 1.8 units, and the cephalin flocculation negative. The total serum protein was 6.8 gm. per 100 cc., albumin 3.7 gm. per 100 cc., and globulin 3.1 gm. per 100 cc. The serologic test for syphilis was negative. The antistreptolysin titer was less than 100 antistreptolysin units per cubic centimeter. Sputum smears and cultures for acid-fast bacilli and respiratory pathogens were negative. Trichinella complement fixation was doubtful. Multiple agglutination tests for Salmonella typhosa O and H Salmonella paratyphi, Brucella abortus, and Proteus OX 19 were negative.

Roentgenologic examination of the chest showed an increase in the interstitial structures of the lungs bilaterally with accentuation of both hilar shadows and evidence of effusion at the right base. The cardiovascular shadow was enlarged in the transverse diameter. Roentgenograms of the upper gastrointestinal tract were negative.

An electrocardiogram taken shortly after admission showed a sinus tachycardia with R-ST segment depression in leads I and II and V_5 and V_6, inverted T in leads I and V_5 and V_6, and absence of R in leads V_1 and V_2. The venous pressure was elevated to 180 mm. of water, and a dehydrocholic acid (Decholin) circulation time was prolonged to 20 seconds. He was treated for congestive heart failure with bed rest, salt-poor diet, digitalis, and mercurial diuretics. There was marked symptomatic improvement with a 10 lb. (4.5 kg.) weight loss in 10 days. Three weeks after admission the spleen tip was barely palpable for the first time, and a soft apical systolic murmur was heard. Serial electrocardiograms showed R-ST segment and T-wave changes toward normal and a decrease in the ventricular rate, although there were many premature ventricular contractions. The hemograms showed a persistent leucocytosis varying from 15,000 to 22,000 cells, with a steadily rising eosinophilia which reached 74%. A sternal marrow aspiration showed only a marked eosinophilia. One skin tissue specimen and two muscle tissue specimens which were removed for biopsy were negative. Two preparations on the venous blood and one on the bone marrow were negative for L. E. cells. Five weeks after admission there developed marked weakness, paresthesias of the right leg, and a persistently elevated temperature ranging from 102 to 103 F. Numerous blood cultures were negative. Stool examinations were negative for ova, parasites, and enteric pathogens. Rectal tissue taken for biopsy for Schistosoma ova was negative. Repeated urinalyses were consistently negative, and blood pressure readings remained within normal limits. The last two weeks of the patient's illness were characterized by progressive weakness, weight loss, and signs of congestive heart failure. On July 19, 1952, the patient complained of severe dyspnea and died suddenly.

The autopsy (eight hours after death) was restricted to the examination of the neck and thoracic organs, the liver, spleen, and kidneys.

Gross Findings.—There were bilateral hydrothorax and hydropericardium. The heart was enlarged (weight 450 gm.), with considerable hypertrophy of the right ventricle. The epicardial surface was faintly granular and opaque grayish brown. The endocardium of each atrium was studded with pinhead-sized slightly raised discrete and confluent yellow foci. Similar lesions were also seen on the mural endocardium of the left ventricle. All valve leaflets and the main coronary arteries were negative. The myocardium of both ventricles showed widespread focal necrosis and larger areas of fibrosis. The latter were more prominent in the apical portion of the left ventricle, where the ventricular walls were thinned out and the trabeculae carnae atrophied. The lungs were greatly increased in weight and on section the parenchyma appeared diffusely fibrotic. Several small subpleural hemorrhagic infarcts were present in the two lower lobes. The spleen was of average size and weight and displayed numerous capsular and subcapsular grayish white nodules varying in size from 0.3 to 1.0 cm. in diameter, often with central necrosis. A few similar nodules could be made out in the congested pulp. The liver and kidneys showed no gross abnormalities. The skin, the oral and nasopharyngeal mucous membranes, and the conjunctivae were negative.

Microscopic Examination.—Microscopic study revealed interstitial myocarditis; focal necrosis, atrophy, and fibrosis of the myocardium; granulomatous and necrotizing angiitis; focal granulomatosis of the heart, kidneys, and spleen; trabeculitis

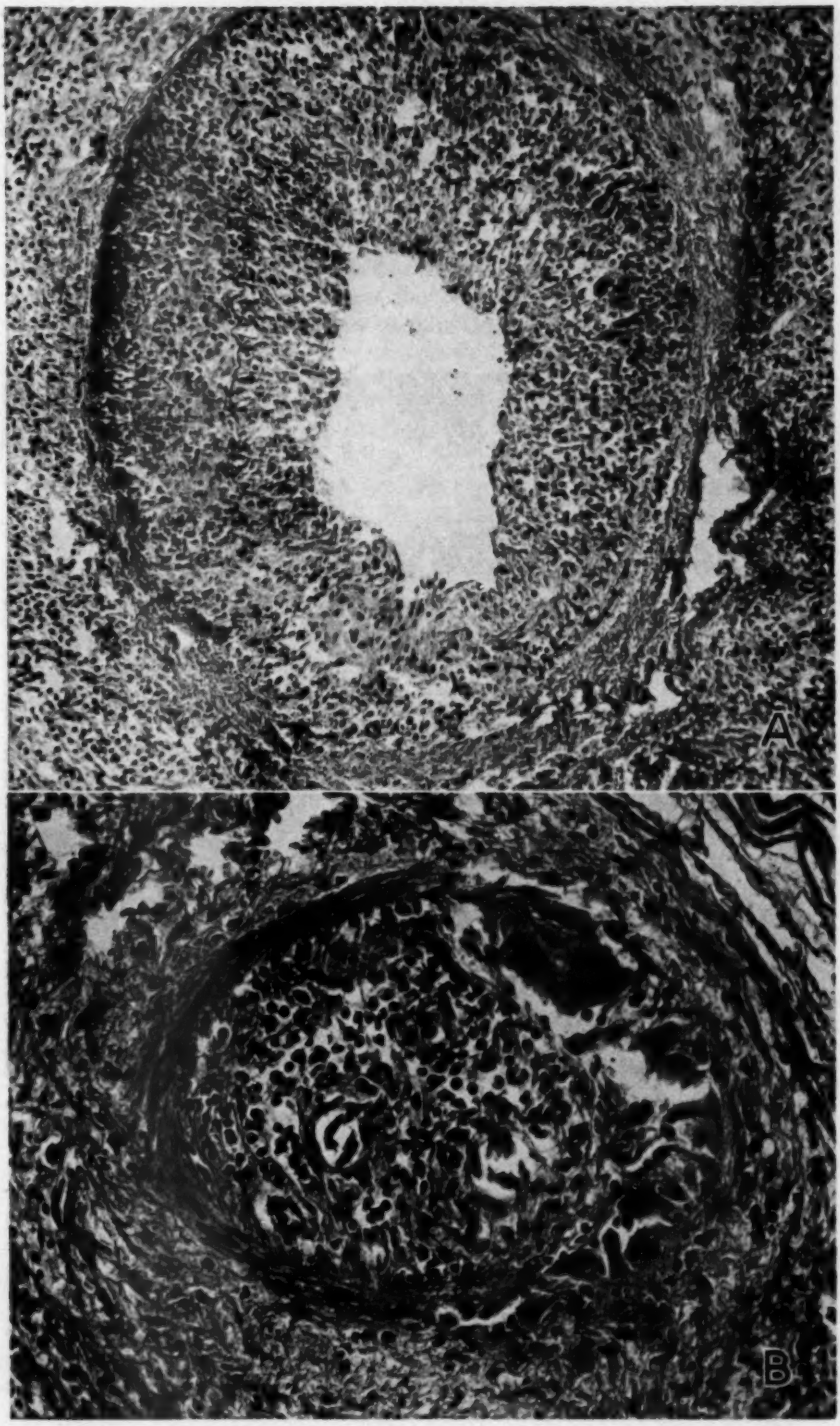

Fig. 1.—*A*, small coronary artery in epicardium. Narrowing of lumen by granulomatous arteritis; necrosis of elastic membranes and band of fibrinoid necrosis in wall at left. Hematoxylin and eosin; × 200. *B*, small coronary artery in myocardium of left ventricle. Granulomatous arteritis occluding lumen; necrosis of part of muscle layer. Hematoxylin and eosin; × 250.

of the spleen, and widely disseminated aggregates of hematoxylin-staining bodies. These features will be described in some detail, with particular emphasis on the hematoxylin bodies. There were no histologic signs of lupus erythematosus.

Blood Vessels: The medium-sized and smaller arteries of the muscular type were affected most conspicuously in the heart and to a less extent in the spleen, liver, esophagus, and lymph nodes. Granulomatous arteritis was seen only occasionally in the small pulmonary arteries and was not found in the kidneys, skin, or skeletal muscle. Fibrinoid necrosis, inflammatory exudate, and granulomas in varying degrees characterized the arterial lesions. These changes commonly

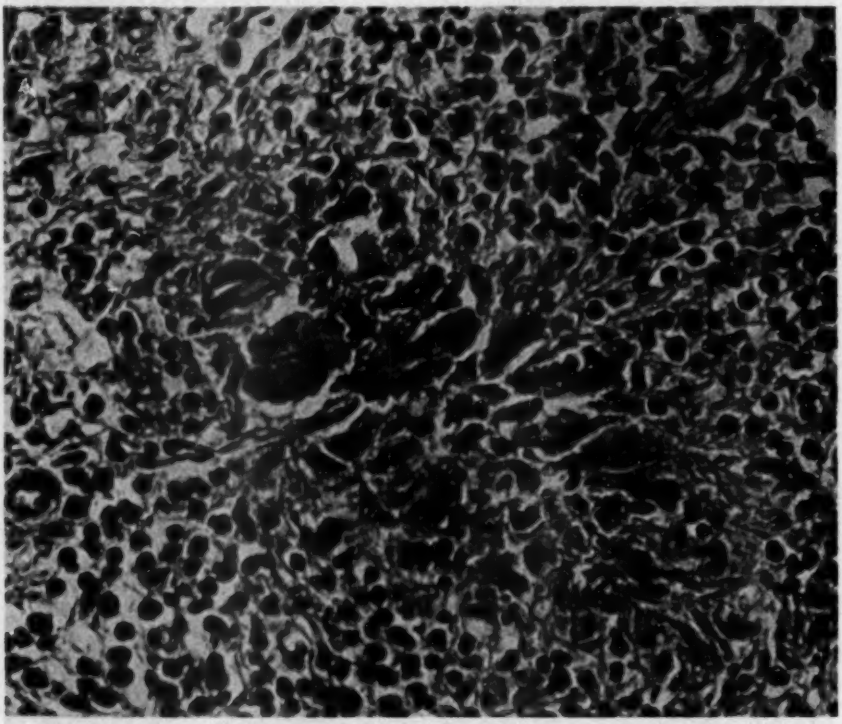

Fig. 2.—Granuloma in myocardium in an area of inflammatory exudate. Epithelioid cells and multinucleated giant cells radially arranged around central focus of necrotic collagen. Hematoxylin and eosin; × 200.

involved all layers and often the entire circumference of the vessel. The granulomatous reaction frequently narrowed the lumens and in some coronary vessels occluded the lumens (Fig. 1). In other vessels extensive fibrinoid necrosis of the walls was accompanied by thrombosis and intense eosinophilic infiltration. Several small arteries in the heart, lymph nodes, and thymic tissue showed advanced sclerosis and greatly narrowed eccentric lumens, changes indistinguishable from that seen in the "healed" stage of periarteritis nodosa.

The veins were similarly affected, though to a far smaller degree. The most advanced granulomatous reaction was seen in the veins in the portal spaces; sub-

intimal granulomas were found in small renal and splenic veins. A number of small epicardial veins exhibited a peculiar basophilic necrosis and fragmentation of their walls. In the hematoxylin and eosin-stained preparations the necrotic walls appeared granular and purplish in color.

Heart: The epicardium was densely infiltrated by a mixture of eosinophilic leucocytes, lymphocytes, and plasma cells which for the most part obscured the epicardial fat. There was a massive interstitial myocarditis in which eosinophiles predominated. In places the collagen of the interstitium showed focal fibrinoid swelling and necrosis. There were widespread focal necrosis, atrophy, and fibrosis of the myocardium, changes due to ischemia secondary to narrowing of the lumens of the small coronary arteries. The main coronary arteries were widely patent. All valve leaflets were normal.

Focal Granulomas: These were most numerous in the heart and were scattered through the epicardium, myocardium, and the endocardium of the two atria (Fig. 2). Frequently they were composed of epithelioid cells and multinucleated giant cells arranged radially around a central focus of necrotic collagen. Similar granulomas were also seen in the kidneys and the spleen.

Lungs: There were diffuse chronic interstitial pneumonitis, fibrotic and edematous thickening of the interlobular septa, and a few small hemorrhagic infarcts. Many of the alveoli and terminal bronchioles were filled with organizing exudate. Scattered throughout the parenchyma were collections of degenerating and necrotic eosinophiles. Some of the smaller bronchi were heavily infiltrated with eosinophiles, and the basement membranes were thickened and hyalinized.

Spleen: The pulp was diffusely infiltrated with eosinophiles which in places were concentrated into dense collections and showed varying stages of degeneration. A number of the trabeculae were disrupted by dense eosinophilic infiltration, and large parts of some trabeculae were entirely replaced by the eosinophiles.

Kidneys: It is noteworthy that the glomeruli and tubules were entirely normal. The interstitium was free of cellular infiltration.

The liver, lymph nodes, skin, skeletal muscle, and diaphragm were negative.

Hematoxylin-Staining Bodies: These appeared as aggregations of ovoid or spindle-shaped dark blue and purple-staining bodies within a finely granular pink matrix (Fig. 3). The hematoxylin bodies were generally about the size of the nucleus of a lymphocyte or slightly larger but were also in the form of smaller deep blue particles embedded in a purplish ground substance. The Feulgen reaction of the hematoxylin bodies was positive. The aggregates were irregular in shape, varied from 0.1 to 0.6 mm. in greatest dimension, and were most numerous in the heart, where they were distributed through the epicardium, myocardium, and interstitium. None were seen in the mitral valve leaflets. They were also encountered in the lungs, spleen, lymph nodes, and perinodal tissue and in the thyroid gland. The hematoxylin bodies did not occur singly but always in small or large clumps. Wherever located, whether in the myocardium, fibrous tissue, fat, or in areas of inflammatory cells, the aggregates were clearly demarcated from the surrounding tissues. The adjacent tissues were well preserved and showed no signs of degeneration or necrosis. In places one had the impression that the hematoxylin bodies were derived from fibroblasts and histiocytes which had undergone a kind of alteration unlike the usual cellular degeneration. But more striking were indications that the hematoxylin bodies often originated from eosinophilic leucocytes,

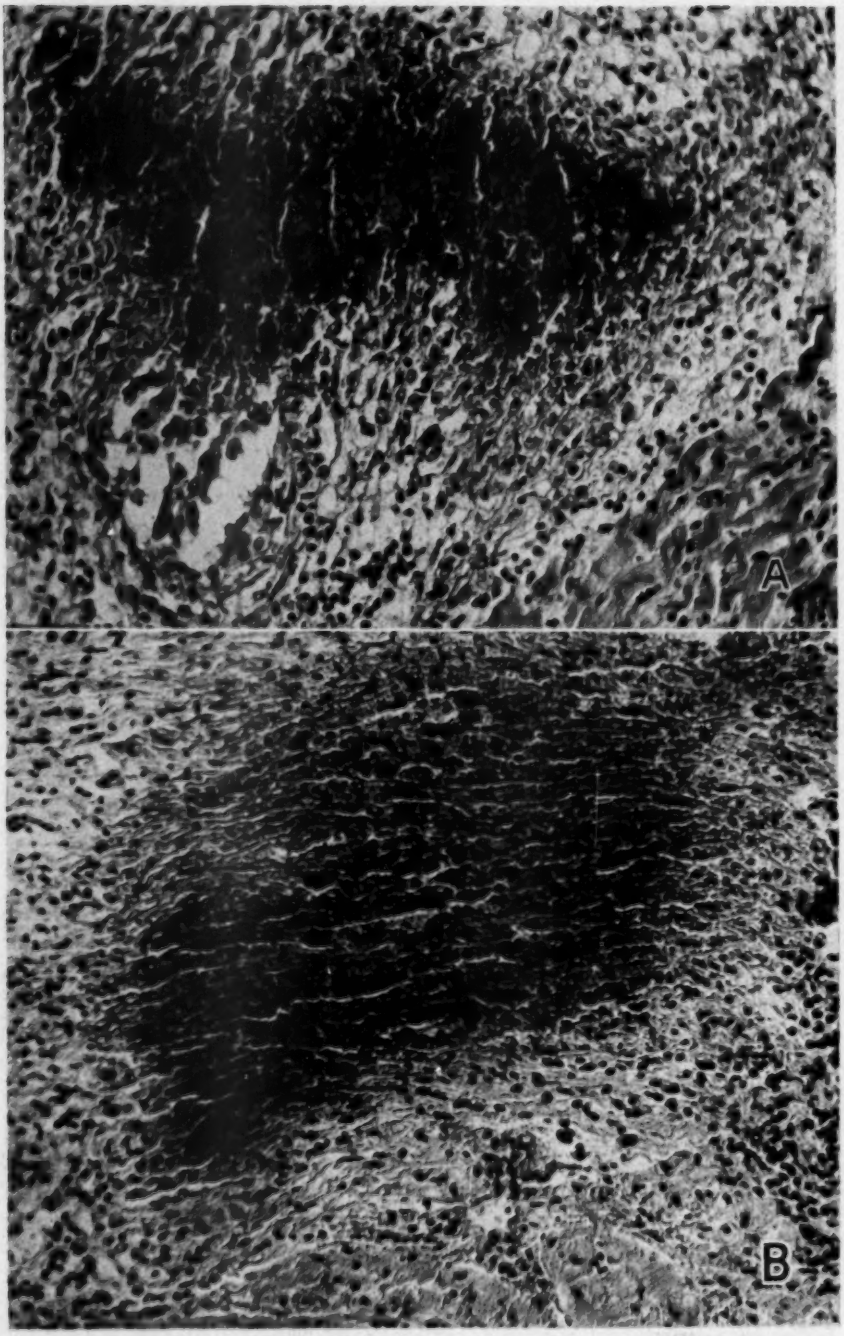

Fig. 3.—*A*, aggregate of hematoxylin bodies in interstitium of right ventricle of heart. Hematoxylin and eosin; × 150. *B*, clump of hematoxylin bodies in myocardium of left ventricle. Hematoxylin and eosin; × 200.

particularly in the areas of eosinophilic exudate. In such areas it appeared as if some eosinophiles became condensed into closely packed masses, seemed to fuse, and in a manner not clear became transformed into the aggregates of hematoxylin bodies described above. It is significant that the surrounding eosinophiles in such areas showed no signs of degeneration, suggesting a peculiar effect on selected groups of cells. Masses of fused blood cells, predominantly eosinophiles, were also seen within the lumens of those epicardial veins with necrotic walls. The intraluminal masses of blood cells in these veins showed transitional changes from one mass to another which appeared to lead to the production of clumps of hematoxylin bodies. Where the vein wall was partly or entirely necrotic, the clumps of hematoxylin bodies were free in the surrounding area, closely adjacent to the damaged vein.

COMMENT

In recent years attention has been called to a fulminant granulomatous angiitis, which is considered a variant of the classical periarteritis nodosa. Principal credit for initiating the segregation of this form of angiitis belongs to Zeek and her co-workers,[3] and to More, McMillan, and Duff.[4] Abundant confirmation has followed their original contributions.[5] This entity which has been designated hypersensitivity angiitis, allergic granulomatous angiitis, and allergic arteritis occurs in association with allergic manifestations and in hypersensitivity to a variety of drugs and antibiotics, particularly the sulfonamides. It is not the purpose of this paper to enlarge upon the vascular lesions and the focal granulomatosis seen in the present case. Recently, Zeek[6] has reviewed the changing concepts in the understanding of vascular disease through the past 50 years and has suggested a classification of periarteritis nodosa and its variants.

Of primary importance, however, was the finding of hematoxylin-staining bodies in the present case. A brief review of the history of these structures is pertinent. First observed by Gross[1] in 1932 in the valvular vegetations in Libman-Sacks disease (a syndrome he later[7] identified with lupus erythematosus), these structures were described as "pyknotic and karyorrhectic masses." However, in 1941, Klemperer, Pollack, and Baehr,[8] in referring to Gross's hematoxylin bodies,

3. Smith, C. C.; Zeek, P. M., and McGuire, J.: Periarteritis Nodosa in Experimental Hypertensive Rats and Dogs, Am. J. Path. **20**:721, 1944. Zeek, P. M.; Smith, C. C., and Weeter, J. C.: Studies in Periarteritis Nodosa: III. The Differentiation Between the Vascular Lesions of Periarteritis Nodosa and of Hypersensitivity, ibid. **24**:889, 1948.

4. More, R. H.: McMillan, G. C., and Duff, G. L.: The Pathology of Sulfonamide Allergy in Man, Am. J. Path. **22**:703, 1946.

5. Rich, A. R.: Hypersensitivity in Disease with Especial Reference to Periarteritis Nodosa, Rheumatic Fever, Disseminated Lupus Erythematosus and Rheumatoid Arthritis, Harvey Lect. (1946-1947) **42**:106, 1947. Allen, A. C.: The Kidney: Medical and Surgical Diseases, New York, Grune & Stratton, Inc., 1951, p. 416. Churg, J., and Strauss, L.: Allergic Granulomatosis, Allergic Angiitis, and Periarteritis Nodosa, Am. J. Path. **27**:277, 1951. Waugh, D.: Myocarditis, Arteritis, and Focal Hepatic, Splenic, and Renal Granulomas Apparently Due to Penicillin Sensitivity, ibid. **28**:437, 1952.

6. Zeek, P. M.: Periarteritis Nodosa: A Critcal Review, Am. J. Clin. Path. **22**:777, 1952.

7. Gross, L.: The Cardiac Lesions in Libman-Sacks Diseases (with a Consideration of Its Relationship to Acute Diffuse Lupus Erythematosus), Am. J. Path. **16**:375, 1940.

8. Klemperer, P.; Pollack, A. D., and Baehr, G.: The Pathology of Disseminated Lupus Erythematosus, Arch. Path. **32**:569, 1941.

disagreed basically with his interpretation of their nuclear origin. They believed that the hematoxylin bodies "were a part of a complex injury whose essential feature is the degeneration of non-cellular components of the collagenous tissue." Since then, Klemperer and his associates [2] have reversed their viewpoint, which now coincides with Gross's original and pioneering observation, thus demonstrating again the difficulties inherent in the accurate interpretation of the changes in this category of diseases.

It is clear from the microscopic observations in the present case that the hematoxylin bodies originated from nuclei of a variety of cell types, predominantly the eosinophiles. It is also apparent that a peculiar and unusual form of cellular degeneration is involved in the production of these structures. The basic nature of this type of cell degeneration remains obscure. Further cytologic investigations may clarify the cellular changes leading to the formation and clumping of these structures. Equally important is the study of postmortem material in a variety of diseases to establish the incidence of the hematoxylin bodies and their relationship to the underlying disease process.

SUMMARY

A case of allergic angiitis in which there were widely disseminated aggregates of hematoxylin bodies is reported. This appears to be the first reported instance in which hematoxylin bodies have been found in a disease other than lupus erythematosus.

Dr. Arthur C. Allen gave his assistance in the interpretation of the histopathologic findings.

SARCOMA OF THE ENDOMETRIAL STROMA

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IN THE course of a recent survey of uterine sarcomas on file at the Armed Forces Institute of Pathology,[1] a total of 92 sarcomas were studied. Of these, 61 were examples of leiomyosarcoma; 16 were classified as carcinosarcoma; 5 were classified as endolymphatic stromal myosis (stromatous endometriosis); 1 was classified as botryoid sarcoma, and 2 were classified as sarcoma arising in the endometrium from the endometrial stroma. There were six cases which could not be classified because of the paucity of diagnostic material. There was one case of so-called "collision tumor" in which a submucous leiomyosarcoma was invaded by an endometrial adenocarcinoma arising around its base. The two cases of sarcoma arising in the endometrial stroma were constributed by the Department of Pathology of Howard University School of Medicine; they furnish the basis for this communication.

Virchow,[2] in 1864, was the first to describe endometrial sarcoma in both its diffuse and its polypoid forms. Most of the cases reported in the following 40 years were summarized by Piquand[3] in 1905; these were reviewed and subsequent ones added by Ewing[4] in 1940. However, the lesion is relatively infrequent, and the occasional case report seldom presents or illustrates histologic details. The incidence of uterine sarcomas of all types in proportion to uterine carcinomas is the ratio of 1 to 40, the majority being leiomyosarcomas arising in leiomyomas. Robert Meyer[5] gives the ratio of endometrial sarcoma to myometrial sarcoma as one to six. His group included a number of varieties, such as mixed mesenchymal tumors, angiosarcomas, and others. Comparatively few sarcomas of the endometrium grow and develop in such a manner as to retain the histologic character of the endometrial stroma, which is the tissue of origin.

From the Department of Pathology, Howard University School of Medicine (Dr. Ober and Dr. Jason), and the Armed Forces Institute of Pathology (Dr. Ober).

1. Grady, H. G., and Ober, W. B.: Carcinosarcoma (Mixed Mesodermal Tumor) of the Uterus, to be published.

2. Virchow, R.: Die krankhaften Geschwülste, Berlin, A. Hirschwald, 1864, Vol. 2, pp. 180 and 350.

3. Piquand, G.: Le sarcome de l'utérus: Etiologie; Anatomie pathologique du sarcome du corps, Rev. gynéc. et chir. adb. 9:387-446, 1905.

4. Ewing, J.: Neoplastic Diseases: A Treatise on Tumors, Ed. 4, Philadelphia, W. B. Saunders Company, 1940.

5. Meyer, R.: Das Schleimhautsarkom, in Henke, F., and Lubarsch, O., editors: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Springer-Verlag, 1930, Vol. 7, Pt. 1, p. 374.

Perry [6] reported a series of 18 uterine sarcomas; only 1 was a typical endometrial stromal sarcoma. Likewise, only one case in Corscaden's [7] series of five of uterine sarcomas falls into this category. MacFarlane,[8] in his experience with 42 uterine sarcomas, had seen only 4 that were endometrial stromal sarcomas, whereas 27 were leiomyosarcomas arising in leiomyomas. Randall [9] noted a higher incidence of endometrial sarcoma, 10 out of 46 cases in his series. However, neither MacFarlane nor Randall presented histologic details in his cases, and it is impossible to evaluate either report.

The classic description of this tumor is that of Robert Meyer,[5] who, like Virchow, described both diffuse and polypoid forms. He pointed out that the sarcoma cells were not much larger than cells of the normal endometrial stroma and that there were no substantial differences in their staining properties. He stressed the value of silver impregnation methods to demonstrate the matrix produced by the sarcoma cells and illustrated both a delicate network of reticulum surrounding individual cells in the diffuse form of the tumor and a coarser meshwork of silver-impregnated fibers in the polypoid form. The value of reticulum stains was later emphasized by Fienberg,[10] who reported two carefully documented cases in which autopsies were performed, demonstrating the polypoid type of this tumor. Both Meyer and Fienberg made a distinction between sarcoma arising from the lamina propria of the endometrium and that arising from the stroma of mucous polyps of the endometrium; they observed a relation of the delicate reticulum fibers to the tumor originating in the lamina propria and of the coarser fibrillary meshwork to the polypoid variety. Moench and Meeker [11] did not make a study of the reticulum in their case of the diffuse variety of the tumor.

McDonald, Broders, and Counseller [12] reported a series of 20 cases of sarcoma of the endometrial stroma, labelling most of them fibromyxosarcoma. This term emphasizes the observation that the tumors were composed for the most part of rather short bipolar cells which resembled fibroblasts and that edema and myxomatous change were present in various areas of the stroma. These authors draw attention to the fasciculated arrangement of the spindle cells and their associated collagen fibers. They confirm the observation of Moench and Meeker [11] that these fibers stain blue with Mallory's aniline blue technique.

Closely related to the endometrial stromal cell sarcoma is the rhabdomyosarcoma arising in the endometrium. This tumor may also be either diffuse or polypoid. A recent report of a case of rhabdomyosarcoma by Kulka and Douglas [13] lists only

6. Perry, T., Jr.: Sarcoma of the Uterus: A Report of 18 Cases, New England J. Med. **238**:793-799, 1948.

7. Corscaden, J. A.: Sarcoma of the Endometrium, Am. J. Obst. & Gynec. **61**:743-754, 1951.

8. MacFarlane, K. T.: Sarcoma of the Uterus: An Analysis of 42 Cases, Am. J. Obst. & Gynec. **59**:1304-1320, 1950.

9. Randall, C. L.: Sarcoma of the Uterus, Am. J. Obst. & Gynec. **45**:445-456, 1943.

10. Fienberg, R.: Endometrial Sarcoma, Arch. Path. **29**:800-812, 1940.

11. Moench, G. H., and Meeker, L. H.: Diffuse Sarcoma of the Endometrium, Am. J. Obst. & Gynec. **30**:435-438, 1935.

12. McDonald, J. R.; Broders, A. C, and Counseller, V S: Sarcoma of the Endometrial Stroma, Surg., Gynec. & Obst. **70**:223-229, 1940.

13. Kulka, E. W., and Douglas, G. W.: Rhabdomyosarcoma of the Corpus Uteri: Report of a Case, Associated with Adenocarcinoma of the Cervix, with Review of the Literature, Cancer **5**:727-736, 1952.

19 previously reported cases. They consider it a "pure" tumor unless it is associated with other mesenchymal derivatives. This tumor may be readily distinguished from the sarcoma of endometrial stromal cells by the presence of the large round or elongated rhabdomyoblasts with their abundant eosinophilic cytoplasm in which longitudinal and/or transverse striations can usually be demonstrated. Neither longitudinal nor cross striations are demonstrable, however, in every rhabdomyoblast, nor are all the cells in these rhabdomyosarcomas fully developed rhabdomyoblasts. Campbell,[14] who reported a case of rhabdomyosarcoma of the uterus and accepted 21 previously reported cases as authentic examples, wrote, "Microscopically the tumor was seen to be composed, for the most part, of rather poorly differentiated cells of variable size." Only a few neoplastic muscle cells were found in his case in proportion to the large number of nondescript cells. This is, in fact, the histologic pattern of many of the tumors classified as rhabdomyosarcomas; the commonest tumor cell is a rather small bipolar cell with comparatively few identifying characteristics. This cell is not distinguishable by morphologic criteria from most of the cells found in endometrial stromal sarcoma. "Round cell" and "spindle cell," often used to define this cell type, are not adequate, for these terms refer to the shape of the nucleus, and most nuclei in any cell, benign or malignant, are round to oval. It is the cytoplasm and the materials deposited within or adjacent to the cytoplasm that characterize the cell functionally. Hence, rhabdomyosarcoma of the endometrium usually represents a special form of the endometrial stromal sarcoma in which some cells have differentiated as neoplastic muscle cells, a few of them exhibiting striations. Relevant to this point is the observation that neoplastic muscle cells are sometimes a component of mixed mesenchymal tumors in the female genital tract and elsewhere, the basic cell, the cell of origin, being a rather primitive mesenchymal cell. Parenthetically, comparison of the list of 21 reported cases of rhadomyosarcoma accepted by Campbell,[14] who added his own to make a total of 22 cases, with the 19 cases accepted by Kulka and Douglas, their own making 20, shows that only 13 cases are common to both lists.

The clinical picture associated with endometrial stromal sarcoma is substantially the same as for the other varieties of uterine sarcoma and has been adequately described by Novak and Anderson,[15] as well as by MacFarlane.[8] It occurs most frequently at or after the menopause, and the usual presenting signs are uterine bleeding, lower abdominal pain, and the presence of a large pelvic mass. The diagnosis is usually established by curettage, and panhysterectomy followed by irradiation is the customary treatment. The prognosis is seldom favorable. A rather high proportion of patients in the earlier case reports died postoperatively. The remainder of the fatalities were due to recurrent or metastatic tumor. Of 15 traced patients from the series reported by McDonald and co-workers, three were alive six years after hysterectomy, and one was alive three years postoperatively. Randall [9] reported a five-year survival of 30% of patients. Presumably, some of the uterine sarcomas are polypoid exophytic growths which do not infiltrate aggressively, and patients with tumors of this type have the better chance for survival.

14. Campbell, C. M., Jr.: Rhabdomyosarcoma of the Corpus Uteri, Arch. Path. **30**:607-613, 1940.

15. Novak, E., and Anderson, D. F.: Sarcoma of the Uterus: Clinical and Pathologic Study of 59 Cases, Am. J. Obst. & Gynec. **34**:740-761, 1937.

REPORT OF CASES

CASE 1 (H. U. S.-49-218, A. F. I. P. Acc. 564296).—A 43-year-old unmarried Negro woman was admitted to the hospital because of persistent pelvic pain and moderately severe menometrorrhagia that had endured for four to six months. She had been under the observation of her physician for several years because of recurrent pelvic pain associated with her menses. Operation had been advised but was declined. Physical examination revealed that the uterus was enlarged and nodular; the cervix was pushed to the right; there was tenderness in both vaults. The remainder of the physical examination was not remarkable. A preoperative diagnosis of endometriosis and leiomyomata uteri was made. Hysterectomy and left salpingo-oophorectomy were performed on Feb. 7, 1949. A "chocolate cyst" in the left adnexal area formed a mass which was adherent to the dome of the bladder, the omentum, and some loops of the small bowel. Several grayish plaques were found on the serosal surfaces of the tubes, ovaries, corpus uteri, and pelvic peritoneum. The uterus was deformed by the presence of a few nodules.

The postoperative course was uneventful, and the patient was discharged from the hospital on the 11th postoperative day. About 13 months later she was admitted to another hospital with signs and symptoms of intestinal obstruction. A colostomy and a biopsy of a nodular peritoneal mass were performed. The patient improved temporarily but died at home about six months later. There was no postmortem examination.

Gross Pathologic Observations.—The specimen from the first operation consisted of a somewhat globular uterus and an irregular piece of tissue to which the Fallopian tube was attached. No tissue resembling ovary was received. The uterus measured 11 by 8 by 5 cm. and was diffusely distorted by ill-defined submucous and intramural masses which were both discrete and confluent. The largest mass projected into the endometrial cavity; it was roughly conical and measured 2.4 cm. in its greatest dimension; its cut surface showed soft homogeneous unstructured whitish gray tissue throughout. The base of the mass was not clearly delimited from the myometrium. The other intramural masses were similar in consistency and texture. The endometrium in the superior half of the uterus was diffusely thickened, and its surface was pebbled. The projecting polypoid mass was part of this diffuse thickening.

The Fallopian tube was 5.2 cm. in length and about 1.0 cm. in diameter, and it was attached to a piece of broad ligament that was irregular in outline and measured 5 by 5 by 2 cm. The serosal surface of the Fallopian tube was somewhat roughened, and on it were a few small plaque-like opaque whitish gray thickenings, none exceeding 0.1 cm. in greatest dimension.

Microscopic Examination.—The uterine tumor consisted of an extensive proliferation of small closely packed round, ovoid, and spindle cells with slightly vesicular nuclei in which the chromatin was evenly dispersed and with relatively scanty faintly eosinophilic cytoplasm. The areas in which the round cells predominated tended to be without pattern; but, where the small short-spindle cells predominated, they were often arranged in poorly formed fascicles, with the long axes of the cells in parallel formation, but there was no tendency toward whorling. Numerous intermediate cell forms, mostly ovoid, were also noted. Incorporated in the tumor were numerous non-neoplastic endometrial glands composed of a single layer of columnar epithelial cells. The endometrial surface of the tumor was covered by a single layer of columnar epithelial cells. At the base of the tumor the proliferated cells formed broad ill-defined fascicles which interdigitated with the myometrial fiber bundles. Pleomorphism and hyperchromasia were slight, and mitotic activity, although noticeable, was not pronounced.

Sections prepared by Mallory's aniline blue method showed many wavy blue-stained fibers associated with the cells, chiefly with the more spindle-shaped cells, where they tended to be arranged in bundles. Silver-impregnated sections displayed different densities of fiber concentration. The fibers surrounding the incorporated non-neoplastic glands were delicate and formed a loose meshwork around individual

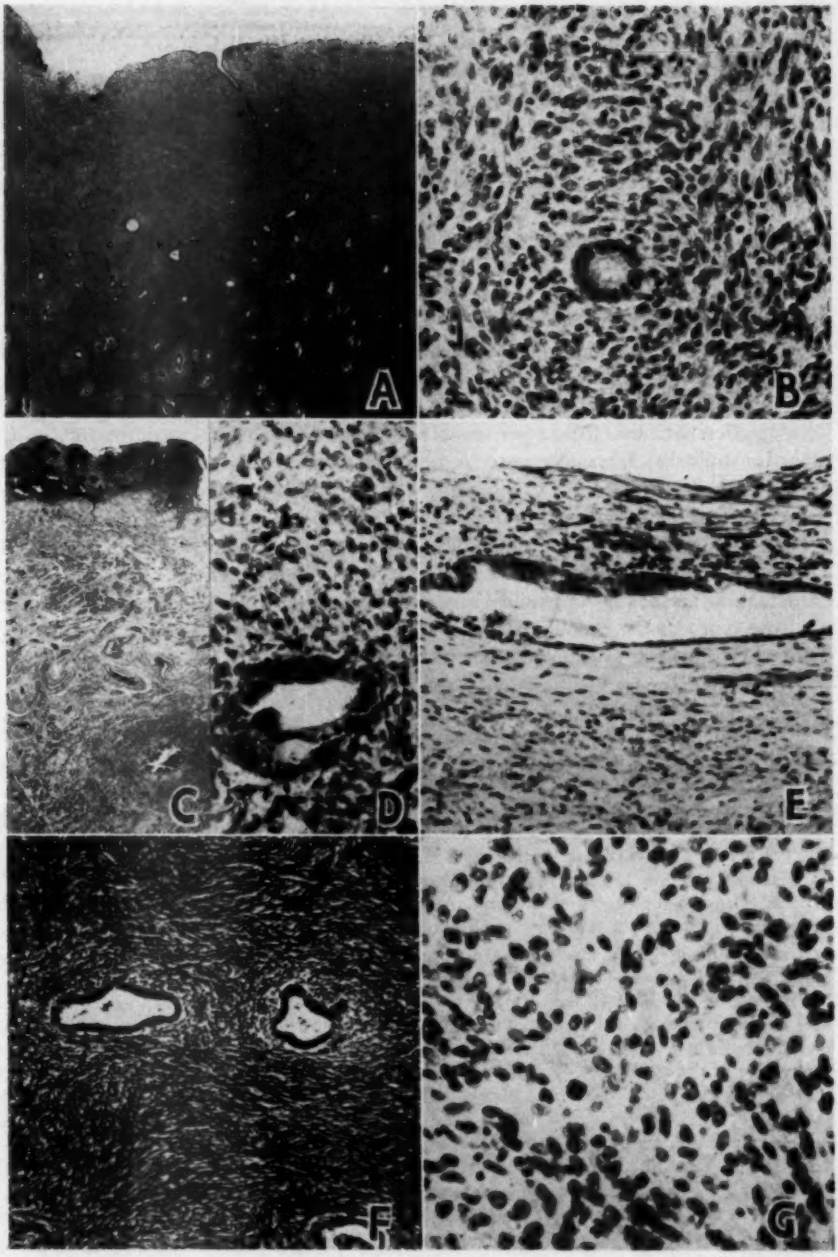

Fig. 1.—Endometrial sarcoma of uterus and Fallopian tube and peritoneal implant (Case 1). *A*, low-power view of sarcoma in uterus; × 15. *B*, neoplastic stromal cells and a non-neoplastic gland; × 185. *C*, sarcomatous transformation of endometriosis on serosa of Fallopian tube; × 15. *D*, detail of Figure 1 C showing sarcoma arising in stroma of endometrial implant; × 185. *E*, endometriosis without sarcoma, opposite margin of Fallopian tube; × 145. *F*, pattern of argyrophilic fibers in relation to stromal sarcoma and non-neoplastic gland. Reticulum stain; × 150. *G*, sarcomatous area in nodule removed at colostomy; × 275.

cells. This meshwork merged gradually with a heavier feltwork of somewhat coarser fibers in the areas of stromal cell proliferation between the glands; in these areas the fibers tended to lie in parallel formation but sometimes were anastomosing, occasionally separating, individual cells or small bundles.

Sections through the Fallopian tube showed the endosalpinx and surrounding myosalpinx to be free from tumor infiltration or embolization. On the serosal surface of the tube, in areas of plaque-like thickening, the pronounced overgrowth of stromal cells was identical in appearance to that described in the uterus. Incorporated among these stromal cells were several non-neoplastic glandular structures resembling endometrial glands. Elsewhere on the surface of the tube were scattered areas of non-neoplastic endometrial type glands surrounded by a scanty spindle cell stroma which could be distinguished from the proliferated stroma in the uterine tumor and in the plaque-like thickenings on the tubal serosa by their smaller cells, less vesicular nuclei, and more orderly arrangement.

A section of tissues from a peritoneal nodule removed at the time of the colostomy was also studied.[16] It was composed of neoplastic round, ovoid, and spindle cells that were identical in appearance to those in the uterine and tubal tumors. The cells were somewhat more hyperchromatic; mitotic figures were more numerous, and the architecture of the tumor tissue not so well organized as in the primary tumor. A few non-neoplastic glands composed of a single layer of cuboidal cells were present in the sarcomatous mass; they resembled endometrial glands. The diagnosis was sarcoma (diffuse type) of the endometrial stroma of the uterus, sarcoma of the endometrial stromal type arising in endometriosis of the fallopian tube and peritoneum.

Case 2 (H. U. S.-52-1436 and S-52-1554, A. F. I. P. Acc. 556311).—A 64-year-old primigravid primiparous Negro woman had noted irregular uterine bleeding for 23 years, ever since the birth of her only child. Before that pregnancy her menses had always been regular. There was no clinical evidence of menopause. During the week before admission to the hospital she had suffered from severe lower abodminal cramps, constipation, and vaginal bleeding. Physical examination revealed a uterus symmetrically enlarged to about three times the normal size. The cervix was that of a parous woman. No masses or tenderness were found in the adnexal area. The remainder of the physical examination was within normal limits.

Dilatation and curettage were performed on Aug. 22, 1952, followed by hysterectomy and bilateral slapingo-oophorectomy on Sept. 8. The patient was free from signs of recurrent or metastatic disease six months after operation.

Gross Pathologic Observations.—The curettings were composed of fragments of pinkish white fleshy tissue and intermingled clotted blood.

The uterus measured 16 by 11 by 9 cm. The serosal surface was smooth and glistening. The corpus uteri was enlarged and globular. The cervix uteri measured 4 cm. in length and 2.5 cm. in diameter; healed lacerations were noted at the lateral angles. No gross lesion was visible at the cervical os. When the uterus was opened the cervical canal was of the usual trabeculated pattern and uniformly smooth. The endometrial cavity was almost entirely filled by a large fleshy polypoid mass, 9 by 7.3 by 4.8 cm., arising from a broad peduncle about 3 cm. in diameter in the right posterior endometrium. The surface of the mass was complexly bossellated. The tips of the polypoid bosses were edematous, and a few were superficially hemorrhagic. For the most part the surface of the mass was smooth, glistening, and pinkish tan, as was the adjacent myometrium. Subsidiary small nodular and plaque-like thickenings in other parts of the endometrium were firmer than the adjacent endometrium, which was somewhat thinned throughout. On section these smaller plaques were of the same color, consistency, and texture as the main tumor, all being moderately firm, unresilient, uniformly

16. Courtesy of Dr. Thomas Peery, George Washington University Hospital, Washington, D. C.

whitish gray, and smooth. Section through the base of the main polypoid mass showed an indistinct plane of demarcation between the base and the myometrial fibers, but there was only superficial infiltration of the mass into the subjacent myometrium. At no point was grossly detectable tumor tissue seen less than 1.8 cm. from the serosal surface. The independent plaques and nodules were confined to the endometrium and did not involve the myometrium. Elsewhere, the myometrium contained a few small firm nodules, measuring 1 to 2 cm. in diameter, their cut surfaces presenting the characteristically whorled appearance of leiomyomas. The myometrial fibers were compressed about these nodules (in distinction to the arrangement of fibers at the base of the pedunculated tumor) and tended to retract from the cut surface. The right Fallopian tube measured 7.6 cm. in length; the left Fallopian tube measured 10 cm.; both tubes were slender, and their lumens were patent. The right ovary measured 3.8 by 2 by 1.2 cm.; the left ovary measured 2.5 by 1.5 by 0.7 cm. Their surfaces were grayish white and corrugated. No nodular deposits were seen outside of the endometrial cavity.

Microscopic Examination.—Sections stained with hematoxylin and eosin showed the tumor to be composed of fairly small relatively uniform cells of two general configurations with numerous intermediate forms. Some of the cells were irregularly round with a round nucleus and a scanty, faintly eosinophilic cytoplasm; other cells were more definitely bipolar with an ovoid nucleus and with similarly staining cytoplasm arranged so that its greatest volume lay at the narrow ends of the nucleus, producing a small spindle-shaped cell. The cell boundaries were indistinct. The nuclei were moderately hyperchromatic, but the chromatin was fairly evenly distributed throughout. The cells resembled closely those of the normal endometrial stroma, and, in blocks taken to include normal endometrium, the transition from non-neoplastic to neoplastic endometrial stroma was gradual. Mitotic figures were seen in some quantity, but abnormal mitoses were rarely encountered. The proliferated round and spindle cells occupied the whole thickness of the endometrium in most areas. The single layer of superficial cuboidal epithelial cells of the normal endometrial surface also covered the areas of cellular proliferation. Included within the substance of the tumor were normal non-neoplastic glands composed of a single layer of columnar cells. In a few places on the surface the cuboidal epithelium gave way to a thin layer of metaplastic squamous epithelium, and some of the incorporated non-neoplastic endometrial glands were distorted. At the margins of the main tumor and the subsidiary tumor plaques, a few of the endometrial glands were hyperplastic and exhibited papillary infolding and heaping up of the columnar epithelium. Sections through the base of the tumor showed that it had infiltrated the myometrium superficially to a depth of 0.1 cm. and that a few fascicles of smooth muscle were enmeshed in the proliferated tumor cells. A similar histologic picture was seen in sections through the small plaques and nodules of tumor. Near the edges of a few of these the tumor did not occupy the entire thickness of the endometrium but compressed the basal endometrial layer between the endometrium and the subjacent myometrium.

Of particular interest was the arrangement of the tumor cells. In some of the superficial areas the cells were widely separated by edema; in other areas they formed a delicate supporting feltwork for the incorporated endometrial glands, while in still other areas they were arranged in poorly defined bundles. The round cells formed the delicate feltwork, and the spindle cells were more likely to group into bundles. Cells of intermediate form were found in and between regions showing both types of architecture. The isolated cells in the endematous areas were of both the rounded and spindle forms, but most striking was the proportion of cells with a rounded nucleus and delicate cytoplasmic processes arranged radially about the nucleus.

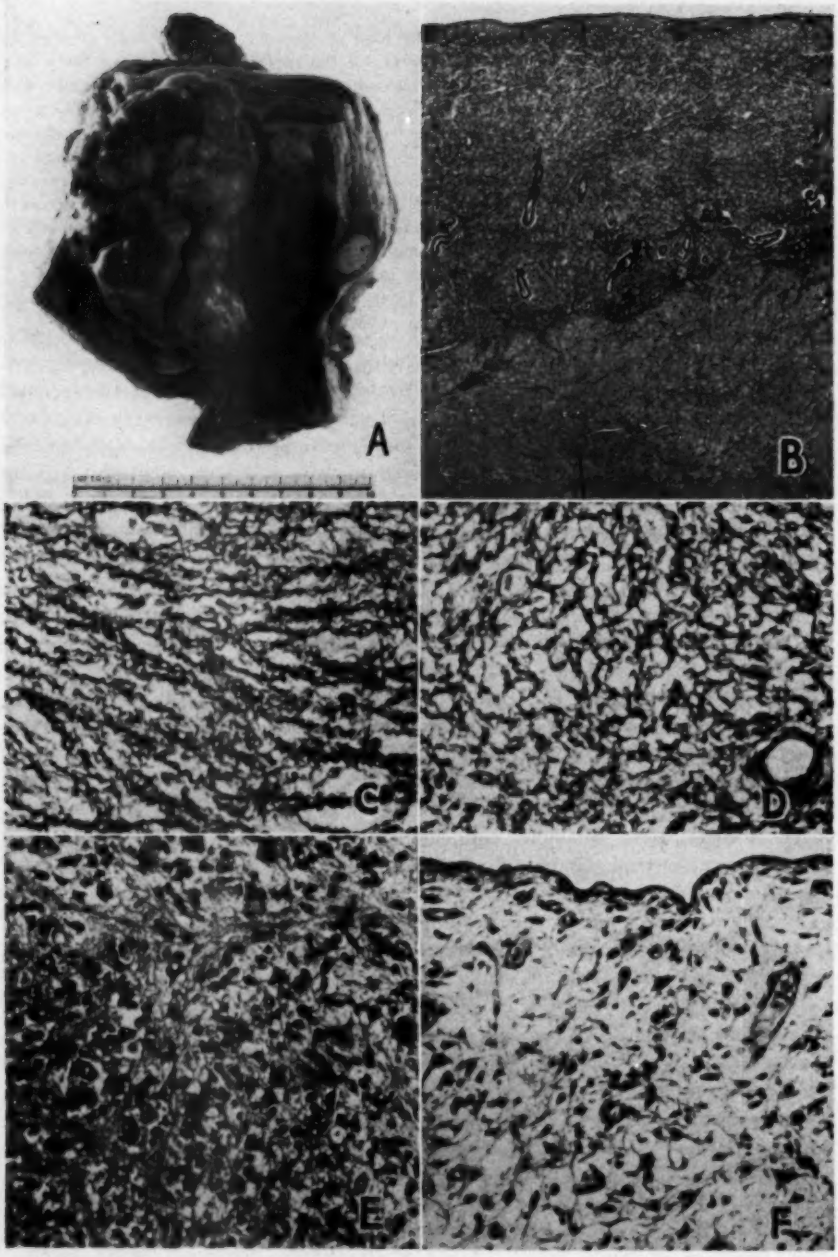

Fig. 2.—Endometrial sarcoma of uterus (Case 2). *A*, sarcoma in endometrial cavity. The uterus has been opened along the anterior midline. *B*, sarcoma lining the uterine cavity. The surface epithelium is squamous. The endometrial glands are non-neoplastic; × 28.5. *C*, argyrophilic fibers in parallel bundles. Reticulum stain; × 350. *D*, argyrophilic fibers surrounding individual cells. Reticulum stain; × 350. *E*, detail to show size, shape, and an arrangement of tumor cells; × 175. *F*, detail to show size, shape, and an arrangement of tumor cells in an edematous area; × 175.

("stellate cells"). Similar cells could not be identified in the nonedematous areas, where cellularity was greater. In a few areas the tumor cells were haphazardly arranged, and here hyperchromasia, pleomorphism, large and irregular nuclei, and mitotic figures were most numerous.

Sections stained by Masson's and Gomori's trichrome methods showed that numerous long narrow fibers were present in areas exhibiting the fasciculated pattern of small spindle cells. These fibers formed branching septa between aggregates of tumor cells and presumably were associated with the polypoid architecture of the tumor; they were absent in the edematous areas, but the Gridley reticulum stain brought out the delicate argyrophilic fibrils surrounding individual tumor cells. The coarser fibrillary matrix, so prominent in aniline blue preparations, was also stained by this method. Iron hematoxylin stains clearly demonstrated the radially arranged branching cytoplasmic processes of the "stellate cells." Mallory's phosphotungstic acid hematoxylin stain did not demonstrate fibroglia or cross striations in any of the tumor cells. Sections treated with Schiff's periodic acid reagent added no further information.

In addition to the tumor and its subsidiary tumors, other lesions noted were several small leiomyomas in the myometrium, a moderate inflammatory reaction in the cervix, and the changes of senile atrophy in the Fallopian tubes and ovaries.

The diagnosis was sarcoma, polypoid type, of endometrial stroma of the uterus.

COMMENT

These two cases of uterine sarcoma are classified as sarcomas arising in the endometrial stroma, the tumor cells exhibiting a degree of differentiation which preserves morphologic resemblance to the endometrial stromal cells. The first case represents the diffuse form of the lesion. Despite its lack of the cytologic features suggesting aggressive malignant behavior, it was widely disseminated and proved to be fatal 19 months after hysterectomy. We are inclined to interpret the anatomic findings in this case as furnishing evidence for a multicentric origin both from endometrial stroma within the endometrium and from the stromal component of endometriosis involving the pelvic peritoneum. The presence of normal non-neoplastic endometrium-like glands in the midst of neoplastic endometrial stroma on the surface of the Fallopian tube and peritoneum would not be expected if these lesions were construed as metastatic deposits. It is difficult to reconcile notions of the behavior of non-neoplastic endometrial glands with their occurrence in the midst of sarcoma that would be metastatic. The presence of endometriosis without neoplastic stromal changes elsewhere on the surface of the same section of Fallopian tube serves to strengthen this impression. Also compatible with this interpretation is the clinical history, which suggests endometriosis of long standing. Gilbert,[17] as well as others, has pointed out the variety of responses of the endometrial stromal cell, whether it is located normally in the endometrium or located ectopically, usually in relation to the pelvic peritoneum.

The second case is an example of the polypoid form of this tumor. Its gross appearance was that of an exophytic mass which had involved the subjacent myometrium only superficially. Although the case is relatively recent, such an exophytic growth is generally suggestive of a better prognosis than the diffuse

17. Gilbert, B.: The Endometrial Interstitial Cell and the Varieties of Growth to Which it May Give Origin, Clin. Proc. **2**:214-221, 1943.

infiltrative form, despite the areas which exhibit the cytologic features usually associated with more aggressive clinical behavior. It is also noteworthy that there is a suggestion of multicentric origin within the endometrial cavity in this case, although implantation by contact of the tumor with the adjacent endometrium cannot be eliminated from consideration.

The histologic observations in these two cases afford a consideration of certain concepts of the behavior of neoplastic endometrial stromal cells. The normal endometrial stroma is definable in terms of certain attributes of its component cells which can be demonstrated by morphologic techniques. These attributes are (1) the general size, shape, arrangement, and staining reactions of the cells; (2) the association of the cells with delicate argyrophilic fibers which form a reticulum about individual cells; (3) the presence of somewhat coarser fibers, usually found separating groups of cells that stain positively with aniline blue; (4) the ability of the cells to respond to hormonal changes, namely, the decidual transformation in response to corpus luteum hormones or to chorionic gonadotropin, and (5) alteration in metachromasia, usually in a regular cycle corresponding to phases of the menstrual cycle, as described by McKay [18] and others. Although the last two functions are not relevant in these two cases, they are mentioned because of their association with and value for identification of endometrium which is ectopically located. In discussing endometrial stroma as a tissue, it must be noted that embryogenetically it differentiates from the Müllerian duct and the subcoelomic mesenchyme; it retains many of the properties of these structures when it undergoes neoplastic changes, and tissues in the adult derived from the same source retain many features in common with the endometrial stroma, namely, the formation of ectopic decidua on the surface of the ovary and elsewhere under the peritoneal mesothelium during pregnancy.

Meyer,[5] Fienberg,[10] and Moench and Meeker [11] are among the observers who noted that certain sarcomas arising in the endometrium exhibited morphologic features which satisfied one or more of the first attributes. Meyer and Fienberg attempted to differentiate between the diffuse and polypoid forms of this tumor by the nature and distribution of the reticulum fibers. In our cases the patterns of the delicate meshwork and of the coarse fibrillary matrix were common to the two cases and did not serve to differentiate the two forms. Likewise, the presence of fibers which exhibited an affinity for aniline blue confirmed the observations of Fienberg and of Moench and Meeker. Whether this implies that these fibers are of diverse nature and hence that the types of cells associated with them are different or merely that a difference in physicochemical affinity exists between fibers of different diameter but of similar composition and origin cannot be determined by the methods employed. So far as the argyrophilic fibers are concerned, the delicate fibers were found in association with cells which exhibited a network type of growth, much like that seen in the normal endometrial stroma in the midproliferative stage; whereas the coarser fibrillary matrix was associated with cells which formed poorly defined bundles and were more spindle shaped. The latter fibers were the ones that stained positively with aniline blue. It is our impression that the pattern of cell growth is of greater determinative significance than any factor affecting the tinctorial properties of the intercellular matrix.

18. McKay, D. G.: Metachromasia in the Endometrium, Am. J. Obst. & Gynec. **59**:875-882, 1950.

The two cases are classified, then, as cases of sarcomas of the endometrial stroma because of origin in the endometrium, conformity with some of the properties of the non-neoplastic stroma, and absence of heterotopic elements (notably, cartilage, bone, fat, or muscle). It is also notable that the endometrial stromal cell sarcoma characteristically contains within its substance well-formed non-neoplastic endometrial glands which exhibit surprisingly little reaction to the sarcoma that surrounds them. Such glandular elements were present in both cases. Had the glands been of neoplastic configuration, a diagnosis of carcinosarcoma of the endometrium would have been made, and the lesions on the tube and pelvic peritoneum in Case 1 would have been interpreted as metastases. Although superficially there is a resemblance to fibrosarcoma and in the edematous areas at the tips of polypoid growths a resemblance to myxosarcoma, the term fibromyxosarcoma, suggested by MacDonald and co-workers,[12] does not seem as explanatory as endometrial stromal sarcoma, in view of the apparent cell of origin of the tumor.

The taxonomic classification of tumors of this type depends upon one's ideas regarding mixed mesodermal tumors and carcinomas of the uterus. In one sense endometrial stromal sarcoma represents the "pure" homologous sarcoma of the tissue. It is quite opposite in this respect to the "pure" rhabdomyosarcoma in this location (Kulka and Douglas) which is putatively heterologous. Mixed mesodermal tumors must be classed midway between these two extremes, for these tumors contain a variety of the forms which mesenchymal derivatives may assume either in ontogenesis or in tumor development. Probably the carcinosarcoma which is composed of adenocarcinoma and endometrial stromal cell sarcoma without heterologous elements represents the "pure" form of cancer of the endometrium as a unitary tissue. However, these diverse labels may represent merely the incompletely separable groups of tumors which, though they vary somewhat in composition, are essentially derived from the endometrium and merely recapitulate in their morphogenesis any one or all of the possible histologic derivatives of the Müllerian duct and the mesenchyme which surrounds it. Although it is useful to separate these tumors for classification, the subclasses are by no means absolutely distinct from each other. The two cases presented in this communication are considered to be taxonomic "type specimens" of the diffuse endometrial stromal sarcoma and of the polypoid endometrial sarcoma, respectively. In making such a classification it is legitimate to question to what extent the nature of the biologic process would be significantly altered if one small mass of cartilage and/or one small focus of unequivocal adenocarcinoma were to have been found in one of the blocks taken for study.

Case Reports

VISCERAL "VIRAL" DISSEMINATION IN A PATIENT WITH BRONCHOGENIC CARCINOMA

Report of a Case with Autopsy Findings

LEO KAPLAN, M.D.
AND
J. B. TULLY, M.D.
LOS ANGELES

THE CLINICAL association of herpetiform (viral) cutaneous disease in patients with malignant disease, especially Hodgkin's disease and lymphatic leukemia, is well known. One group estimates the occurrence of herpes zoster in women with carcinoma of the breast at 4%.[1] The literature reporting this coexistence fails to mention a single instance wherein visceral lesions of these ectodermotropic viral diseases are described. The occurrence of widespread visceral involvement in such viral infections without simultaneous neoplasms has been recognized in recent years, and fatalities have been described.

The purpose of this report is to present the autopsy findings in a case of bronchogenic carcinoma associated with a disseminated cutaneous and visceral inclusion disease. The latter's distribution played a major role in the cause of death. The morphologic character of the lesions was similar to that of lesions induced by the viruses of herpes simplex, herpes zoster, and varicella.

REPORT OF CASE

The patient was a 61-year-old white man who was admitted to the Veterans Administration Center, Los Angeles, on Dec. 18, 1951, with the chief complaints of increasingly severe dyspnea and a cough productive of foamy mucus that had endured for two and one-half years. A chest roentgenogram revealed prominence of the right hilar mediastinum and a lesion within the base of the upper lobe of the right lung. An exploratory thoracotomy performed on Jan. 7, 1952, revealed an inoperable adenocarcinoma of the upper lobe of the right lung and hilar lymph nodes. At surgery, 150 cc. of serous pericardial fluid was aspirated. Postoperatively the patient did well until Jan. 17, when he began to complain of severe right chest pain and pronounced dyspnea. At the same time a rash was observed on the trunk. On Jan. 18 he complained of abdominal "distress" and on Jan. 19 of abdominal "pain." During the three days following the onset of the eruption, successive crops of lesions appeared on the scalp, trunk, and upper arms. These started as maculopapules and progressed to vesicles. There were generalized cutaneous edema and marked redness of the tongue. On Jan. 19 he became cyanotic and extremely dyspneic. His temperature was 100.6 F. (rectal); pulse rate was 120 to 156 per minute, and respirations were 34 per minute. At 10:00 P.M. the pulse became irregular; the skin was cold and clammy, and diaphoresis was profuse. He became unconscious and died at 8:00 A.M. on Jan. 20, 1952.

From the Laboratory Service, Veterans Administration Center, and the Department of Pathology (Dr. Kaplan), University of California at Los Angeles School of Medicine.

1. Huguenin, R.; Fauvet, J., and Piérart, A.: Les zonas des cancéreux, Bull. et mém. Soc. méd. hôp. Paris **66**:1116-1120, 1950.

An autopsy was performed approximately 24 hours after death. There was a hemorrhagic vesicular eruption over the trunk, arms, and scalp. The greatest number of lesions were discrete reddish brown nonumbilicated vesicles (0.4 to 1.0 cm. in diameter) arising from an erythematous base. The vesicles contained hemorrhagic or clear-colored fluid, and some were covered by brownish eschars. The lesions were confluent at the recent thoracotomy scar.

The visceral and parietal surfaces of the pericardial sac were covered by a diffuse fibrinous adhesive pericarditis. An ovoid firm gray-yellow neoplastic lesion (5.0 cm. in diameter) arose in a small bronchus at the peripheral and basilar portions of the upper lobe of the right lung. A similar tumor involved the adjacent mediastinal lymph nodes and invaded the azygos vein. No other gross or microscopic foci of neoplasm were found in the body. In the pancreas a number of very minute gray-brown foci of necrosis were scattered throughout the body and tail.

The skin lesions disclosed prominent intraepidermal vesiculation with the formation of unilocular or multilocular cavities. The vesicle roofs were composed of the hornified and granular epithelial layers, and the degenerating basal cells or superficial corium constituted the floors. The marginal epithelial layers exhibited moderate swelling, vacuolar degeneration, and detachment of cells into the vesicular spaces. The fluid-filled cavities contained many desquamated swollen and nonswollen epithelial cells, multinucleated giant epithelial cells, erythrocytes, mononuclear cells, and few polymorphonuclear leucocytes.. The upper corium showed marked edema, slight hyperemia, extravasation of blood, and mild infiltration by many polymorphonuclear leucocytes and few lymphocytes.

The marginal degenerating or desquamated epithelial cells of these vesicles frequently revealed slightly distended irregular nuclei. Their chromatin was adherent to the inner aspect of the nuclear membrane. The membranes were thereby thickened and internally beaded. Within the center of these nuclei were found ovoid or round, homogenous or finely granular, eosinophilic or lightly basophilic inclusion bodies. These ranged from 2.4 to 6.0 μ (average 4.1 μ) in size. Between the inclusions and the marginal clumped chromatin, a clear ring-like zone was found. The latter rarely contained minute granules of chromatin or azurophilic particles. At times the clear zones were extremely narrow or absent, and the inclusions then filled the spaces. Identical inclusion bodies were present in the nuclei of the secretory elements of some sebaceous glands in close proximity to the vesicles.

The organizing fibrinous pericardial exudate was pervaded by mesothelial cells, histiocytes, lymphocytes, plasmocytes, and polymorphonuclear leucocytes. Occasionally swollen mesothelial cells contained ovoid, elongated, or slightly irregular intranuclear inclusion bodies. These ranged from 3.6 to 6.0 μ (average 4.4 μ). The interstitial and perivascular connective tissue of the myocardium was moderately edematous.

The upper lobe of the right lung showed an adenocarcinoma with simplex features; this invaded the parenchyma. The uninvolved lung tissue was edematous. Here, the alveolar septa exhibited swelling, smudginess of markings, interstitial edema, fibrin deposition, capillary dilatation, and an infiltrate of occasional polymorphonuclear leucocytes and lymphocytes. The alveolar septal lining cells were large and numerous. The alveoli contained a fibrinous exudate sprinkled with alveolar macrophages and polymorphonuclear leucocytes. Bronchial epithelial cells, alveolar lining cells, macrophages, and occasional carcinoma cells contained intranuclear inclusions similar to those previously described. However, infrequently they were elongated or dumbbell-shaped. On rare occasions multiple inclusions in the same nucleus were found. The inclusions ranged from 2.4 to 4.8 μ (average 4.0 μ). Few round or oval eosinophilic finely granular intracytoplasmic inclusions

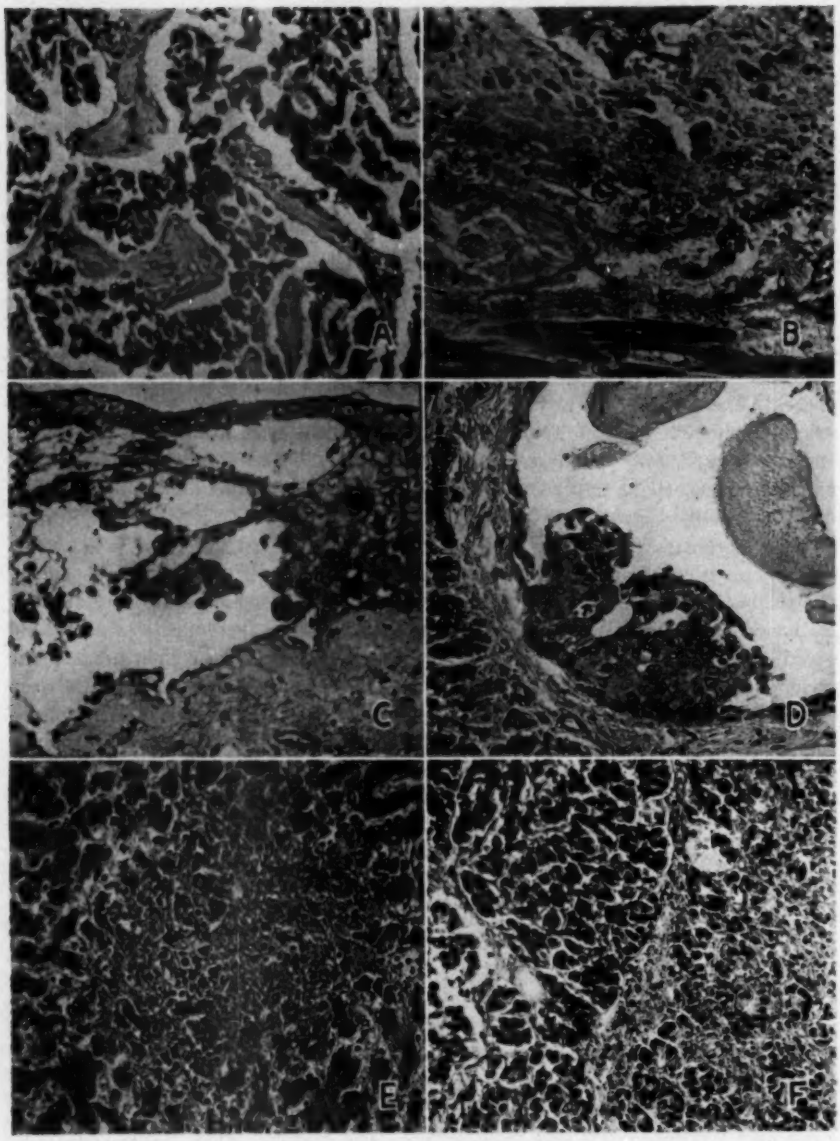

Fig. 1.—*A*, adenocarcinoma of the lung with simplex features. Hematoxylin and eosin; × 120. *B*, diffuse fibrinous pericarditis. Hematoxylin and eosin; × 120. *C*, cutaneous vesicle. Hematoxylin and eosin; × 120. *D*, ductal squamous cell metaplasia; pancreas. Hematoxylin and eosin; × 120. *E*, focal necrosis; liver. Hematoxylin and eosin; × 120. *F*, focal necrosis; pancreas. Hematoxylin and eosin; × 120.

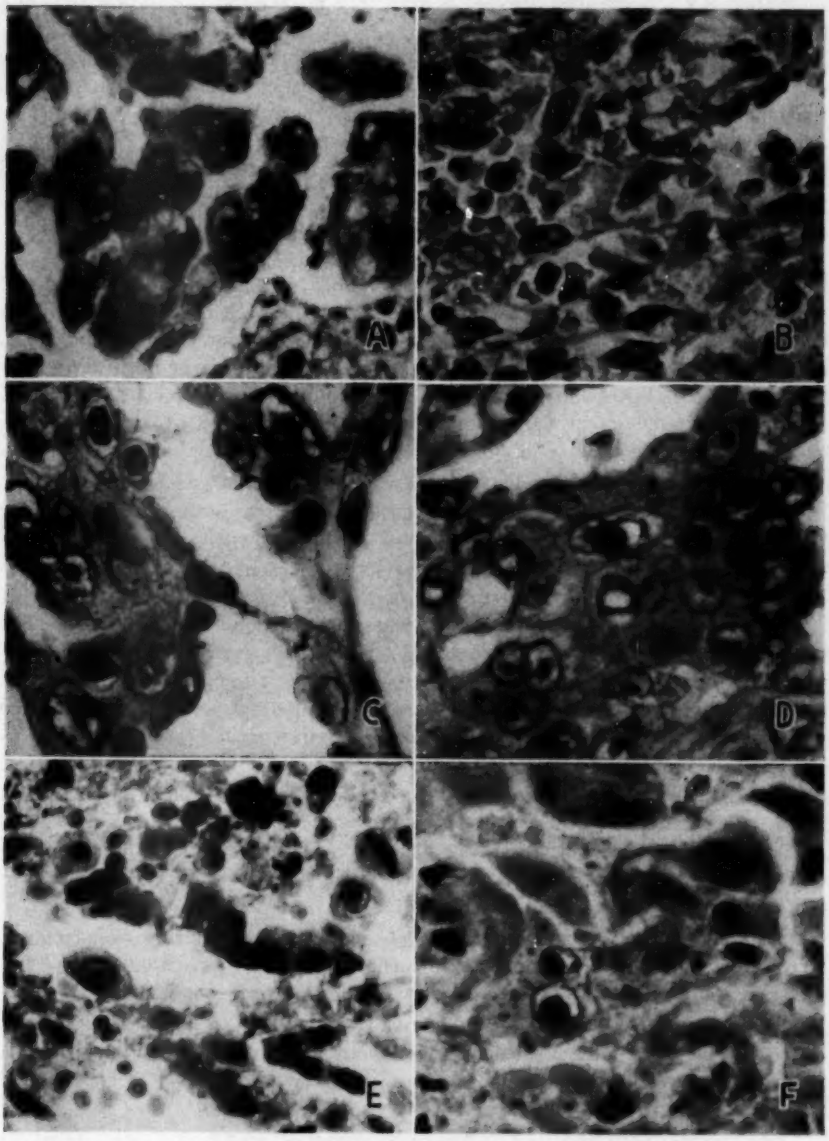

Fig. 2.—*A*, intranuclear inclusion bodies within carcinoma cells; lung. Hematoxylin and eosin; × 700. *B*, intranuclear inclusion bodies within reticuloendothelial cells; spleen. Hematoxylin and eosin; × 700. *C*, intranuclear inclusion bodies within epithelial cells of skin vesicle. Hematoxylin and eosin; × 700. *D*, intranuclear inclusion bodies within metaplastic squamous epithelium; pancreatic duct. Hematoxylin and eosin; × 700. *E*, intranuclear inclusion bodies within desquamated alveolar histiocytes; lung. Hematoxylin and eosin; × 700. *F*, intranuclear inclusion bodies within necrotic liver cells. Hematoxylin and eosin; × 700.

measuring 2.4 μ in greatest dimension were seen in occasional neoplastic cells and alveolar histiocytes.

The liver revealed congestion and a number of small, easily discerned, sharply demarcated foci of amorphous pink granular necrosis. These were located in the midzonal or paraportal portions of lobules. These necrotic foci were infiltrated by a few lymphocytes and degenerating polymorphonuclear leucocytes. The Kupffer and hepatic cells within or immediately adjacent to these necrotic foci contained intranuclear inclusions. These ranged from 3.6 to 6.0 μ (average 4.6 μ) in size. Such cells exhibited mild swelling and degenerative changes. Bile duct epithelium, unrelated to areas of necrosis, also revealed occasional intranuclear inclusions.

The splenic sinusoids were congested. Small, irregular, poorly discerned and poorly defined foci of necrosis, located chiefly in the vicinity of the penicillium arteries were observed. These foci were pervaded by a few inflammatory cells. Many histiocytes and sinusoidal reticuloendothelial cells within or in close proximity to the necrosis contained intranuclear inclusion bodies which ranged from 2.4 to 6.0 μ (average 4.4 μ) in size. The cells and nuclei involved by such inclusions rarely exhibited an increase in either nuclear or total-cell size.

The architecture of a hilar lymph node was completely effaced as a result of diffuse neoplastic invasion. Intranuclear inclusions were occasionally noted within the tumor cells, fibrocytes, and the smooth muscle cells of a small caliber artery. The inclusions measured from 3.6 to 4.8 μ (average 3.7 μ).

The largest necrotic foci were those of the pancreas. These were minimally infiltrated by lymphocytes and degenerating polymorphonuclear leucocytes. The pancreatic ducts near the areas of necrosis revealed prominent squamous cell metaplasia and cystic dilatation. Intranuclear inclusions were found within the necrotic tissue, normal and squamous metaplastic ductal epithelium, exocrine parenchymal cells, and the endothelium and smooth muscle of small caliber vessels. The inclusions ranged from 2.4 to 8.4 μ (average 5.1 μ).

The cortices of the adrenal gland showed prominent hyperplasia. An occasional small, poorly defined focus of necrosis, mildly invaded by inflammatory cells, was present in the cortex. The intranuclear inclusions involved some of the parenchymal cells within and adjacent to the degenerative foci. They ranged from 3.6 to 6.0 μ (average 4.9 μ) in greatest dimension.

Renal tubular epithelial cells, intact, as well as intraluminally desquamated, were rarely involved by the intranuclear inclusions. They ranged from 2.4 to 6.0 μ (average 4.8 μ) in size. There was no evidence of necrosis or inflammation.

Small irregular, ill-defined, smudgy edematous foci were observed within the hematopoietic tissue of a lumbar vertebral body. A few reticuloendothelial cells within these areas contained elongated or irregular intranuclear inclusions that ranged from 2.4 to 8.4 μ (average 4.8 μ).

The thyroid gland, testis, prostate gland, pituitary gland, and brain failed to reveal significant pathological change.

REVIEW OF LITERATURE

The accessible literature revealed a limited number of fatal cases with visceral involvement induced by herpes simplex and varicella. Fatalities resulting from herpes zoster were not recorded. This review does not include those cases in which

the lesions induced by the spread of the virus was restricted to the central nervous system.

In 1927, Schleussing [2] described premature male twins who acquired varicella in the third week of life and died without obvious complications. He observed foci of necrosis in the liver, spleen, and adrenal glands, but apparently did not discern inclusion bodies. When, in 1935, Haas [3] found hepatic and adrenal necrosis associated with intranuclear inclusion bodies of the Type A (Cowdry) variety in a 2-week-old premature Negro girl, he suspected a herpetiform disease despite the absence of cutaneous involvement. Johnson,[4] in 1940, reported an instance of varicella occurring in a 7-month-old boy in whom the skin and viscera showed changes consistent with viral injury and the presence of typical intranuclear inclusions. In 1942, Waring and co-workers [5] presented a detailed account of a fatal varicella pneumonitis in a 40-year-old white man. They noted intranuclear and intracytoplasmic inclusions in the tracheal epithelium. However, their description was brief, and the character of the inclusions could not be ascertained. The skin lesions were not examined. An autopsy performed by Oppenheimer [6] in 1944 on an 11-day-old premature girl with varicella revealed widely disseminated lesions with characteristic intranuclear inclusions in the skin and involved viscera. Lucchesi and co-workers,[7] in 1947, noted the typical intranuclear inclusions in the skin, lungs, liver, and stomach of a 12-day-old premature girl with varicella. Although Claudy,[8] in 1947, found pneumonitis, as well as hepatic and splenic necrosis, in a 32-year-old white woman with varicella, his description of the intranuclear inclusions observed in the skin and liver were not morphologically distinctive. Frank,[9] in 1950, reported an autopsy on a 34-year-old white woman who died of varicella pneumonitis. The cutaneous and pulmonary lesions contained Type A intranuclear inclusions. The virus of herpes simplex was isolated from the skin scrapings of a 2-week-old boy by Quilligan and Wilson [10] in 1951. Classic intranuclear inclusions were found in the hepatic lesions. A 70-year-old white woman with Boeck's sarcoidosis succumbed to a widespread varicella infection, as stated in the case report of Eisenbud [11] in 1952. He discovered the characteristic intranuclear

2. Schleussing, H.: Nekrosen im Leber, Milz und Nebennieren bei nicht vereiterten Varizellen, Verhandl. deutsch. path. Gesellsch. **22**:288-292, 1927.

3. Haas, G. M.: Hepato-Adrenal Necrosis with Intranuclear Inclusion Bodies, Am. J. Path. **11**:127-142, 1935.

4. Johnson, H. N.: Visceral Lesions Associated with Varicella, Arch. Path. **30**:292-307, 1940.

5. Waring, J. J.; Neuberger, K. T., and Geever, E. F.: Severe Forms of Chickenpox in Adults, with Autopsy Observations in Case with Associated Pneumonia and Encephalitis, Arch. Int. Med. **69**:384-408, 1942.

6. Oppenheimer, E. H.: Congenital Chickenpox with Disseminated Visceral Lesions, Bull. Johns Hopkins Hosp. **74**:240-250, 1944.

7. Lucchesi, P. F.; La Boccetta, A. C., and Peale, A. R.: Varicella Neonatorum, Am. J. Dis. Child. **73**:44-54, 1947.

8. Claudy, W. D.: Pneumonia Associated with Varicella: Review of Literature and Report of Fatal Case with Autopsy, Arch. Int. Med. **80**:185-192, 1947.

9. Frank, L.: Varicella Pneumonitis: Report of Case with Autopsy Observations, Arch. Path. **50**:450-456, 1950.

10. Quilligan, J. J., Jr., and Wilson, J. L.: Fatal Herpes Simplex Infection in a Newborn Infant, J. Lab. & Clin. Med. **38**:742-746, 1951.

11. Eisenbud, M.: Chickenpox with Visceral Involvement, Am. J. Med. **12**:740-746, 1952.

inclusions not only in the skin and many viscera but also within the sarcoid nodules. A group of eight infants with fulminating herpetiform visceral disease was presented by Zuelzer and Stulberg [12] in 1952. In every case, one or more viscera contained intranuclear inclusion bodies. The herpes virus was isolated in only one case.

This is the extent of the available reports of visceral involvement in the fatal cases of herpes simplex and varicella infections. This represents only 9 cases of varicella and 10 cases of herpes simplex. Those with varicella were four premature infants whose ages ranged from 11 to 21 days, one 7-month-old child, and four adults whose ages ranged from 32 to 70 years. Those with herpes simplex were five premature children whose ages ranged from 5 to 14 days, three full-term children ranging from 7 to 21 days, and two children of 18 and 22 months of age.

Viscera Involved in Order of Decreasing Frequency

	Varicella					Herpes Simplex						Case Report	
Organs	Proved*	Probable†	Inclusions Described	Total No. of Cases	Footnote No.	Proved*	Inclusions Described	Probable†	Inclusions Described	Total No. of Cases	Footnote No.	Probable†	Inclusions Described
Liver	6	7	4	7	2, 4, 6, 7, 8, 11	2	2	8	4	10	3, 10, 12	1	1
Skin	0	9	5	9	2, 4, 5, 6, 7, 8, 9, 11	2	0	3	1	5	10, 12	1	1
Spleen	0	6	2	6	2, 6, 8, 9, 11	1	0	5	0	6	12	1	1
Adrenal gland	0	5	3	5	2, 4, 6, 11	1	1	5	1	6	3, 12	1	1
Lung	0	6	4	6	5, 6, 7, 8, 9, 11	1	1	3	0	4	12	1	1
Gastrointestinal tract	0	4	3	4	4, 6, 7, 9	1	1	5	2	6	12	..	..
Kidney	0	2	2	2	4, 6	1	1	3	1	4	12	1	1
Lymph nodes	0	1	1	1	11	1	0	3	1	4	12	1	1
Brain	0	1	1	1	5	1	1	3	1	4	12	..	..
Bone marrow	0	1	0	1	6	1	0	3	0	4	12	1	1
Pancreas	0	2	2	2	4, 6	..	..	..	..	..	..	1	1
Tongue	..	..	..	..	..	1	1	2	2	3	12	..	..
Conjunctivae	..	..	..	..	..	1	0	2	0	3	12	..	..
Bladder and ureters	0	1	1	1	4	..	..	..	..	..	..	..	..
Thymus	0	1	1	1	6	..	..	..	..	..	..	..	..
Pericardium	..	..	..	..	..	..	..	..	..	..	..	1	1

* Isolation of a virus.
† No virus study or virus isolation.

Of those who succumbed to varicella, four were males and five were females; while with herpes simplex, five were males and five were females.

Approximately half of the 19 cases demonstrated a specific clinical pattern. With varicella, four presented a prominent pulmonary disease, and one presented a primary hepatic dysfunction. With herpes simplex, there were three cases that presented a pulmonary disease; another presented significant cerebral signs, and one presented dominant gastrointestinal symptomatology.

The prominence of clinical involvement of one organ stems from the propensity for visceral localization that frequently overshadows all other involved systems.

The widespread visceral involvement in these diseases is illustrated in the Table. A comparison between varicella and herpes is made to demonstrate the fre-

12. Zuelzer, W. W., and Stulberg, C. S.: Herpes Simplex Virus as the Cause of Fulminating Visceral Disease and Hepatitis in Infancy: Report of Eight Cases and Isolation of the Virus in One Case, A. M. A. Am. J. Dis. Child. **83**:421-439, 1952.

quency of organ localization of virus and to show the number in which inclusions were specifically described.

In only five cases was an attempt made to establish the identity of the causative virus. Viral isolation was successful in two cases of herpes simplex and unsuccessful in three cases of varicella. Where isolation studies were successfully accomplished, the cases were designated as "proved." All other reported cases were recorded as "probable."

The reliability of such data and its usefulness in drawing significant conclusions is limited. In our experience the visualization of the characteristic intranuclear inclusions was not easy, even when the presence of virus dissemination was clear-cut. In the reported case, intranuclear inclusions were found by us in certain locations only after many successive studies had been made. This was particularly true for those organs, such as the kidney, pericardium, hilar lymph node, and bone marrow, in which involvement was not sign-posted by forthright necrosis. The reported cases from the literature, almost without exception, described intranuclear inclusions in relationship to foci of visceral necrosis. In addition, some of the authors failed to clearly describe the type of inclusions they observed, or they neglected to emphasize their exact location within the involved viscera, making evaluation extremely difficult or impossible.

REVIEW OF CASE REPORT

The case reported revealed the dissemination of a disease producing a variety of histopathological changes in various organs, the most outstanding of which was the production of an intranuclear inclusion body. Morphologically its similarity to those induced by known infections with the viruses of herpes simplex, herpes zoster, and varicella was significant; however, the appearance of the inclusion bodies produced by this disease was not a point of differentiation. In the skin a diffuse vesiculating eruption characterized the infection. In such organs as liver, spleen, pancreas, and adrenal glands, foci of necrosis were prominent. In the lungs the "virus" accounted for the presence of a severe, acute, interstitial pneumonitis and an alveolar exudate rich in histiocytes. In the pericardium a diffuse fibrinous pericarditis occurred. In such organs as the kidney, hilar lymph node, and bone marrow, infection by the "virus" was merely marked by intranuclear inclusions and minimal fixed tissue reaction.

The "virus" inclusions were primarily intranuclear in the skin, liver, spleen, adrenal gland, lung, kidney, lymph nodes, bone marrow, pancreas, and pericardium. However, in the lung, smaller intracytoplasmic inclusions were found in occasional alveolar histiocytes and adenocarcinoma cells. In the latter cells, intranuclear inclusions morphologically identical to the others were also seen. The intracytoplasmic inclusions were usually smaller and ranged about 2.4 μ in diameter, whereas the intranuclear inclusion bodies were generally larger and ranged from 3.7 μ in the hilar lymph nodes to 5.1 μ in the pancreas. The average inclusion size for all organs was 4.5 μ in diameter.

The "virus" inclusions involved epithelial and mesenchymal tissues. The stratified squamous cells and secretory epithelial elements of sebaceous glands of the skin, the bronchial epithelium, the alveolar lining "epithelial" cells and adenocarcinoma

cells of the lung, the duct epithelium and parenchymal cells of the liver and pancreas, the adrenal cortical cells, and the tubular epithelial cells of the kidney were all involved by inclusions. The mesenchymal tissues found to contain inclusions were the mesothelial cells and histiocytes of the pericardial exudate, the intra-alveolar "histiocytes" of the lungs, an occasional fibrocyte of the hilar lymph node, the smooth muscle of small arteries in the hilar lymph node and pancreas, and the endothelial cells of the liver, spleen, lymph nodes, pancreatic blood vessels, and bone marrow.

Although the chief diagnosis of the reported case was quite clearly that of pulmonary carcinoma, the likelihood of a coexisting generalized viral infection was not entertained at any time prior to necropsy. Unfortunately, attempts to recover the etiologic "viral" agent were not made, and the exact type of "virus" disease must remain obscure.

COMMENT

No real difference in organ involvement appears to exist for varicella or herpes simplex. The failure to observe pancreatic inclusions in infections with herpes simplex is striking since the organ is fairly regularly studied during routine autopsies. However, the failure to observe inclusions in such tissues as tongue, conjunctiva, bladder, ureter, thymus, and pericardium is not surprising since they are generally studied only when gross lesions draw the prosector's attention to them. In varicella the four organs most frequently involved are the skin, liver, lung, and spleen; while for herpes simplex they are the liver, gastrointestinal tract, spleen, and adrenal gland. In the Table a statistical difference that remains to be explained is the involvement of the liver in all (100%) of the reported cases of herpes simplex and in 77% of the cases of varicella, while cutaneous involvement occurs in all (100%) of the fatal cases of varicella and in only 50% of those cases of disseminated herpes virus infection.

In this case the observation of intranuclear inclusions within the pericardial mesothelial cells was unique. No sections of the gastrointestinal tract, tongue, conjunctiva, bladder, ureter, and thymus were taken for study.

Histologically identical intracellular inclusion bodies can be found in the lesions of herpes simplex, herpes zoster, and varicella. These are predominantly intranuclear inclusions.[13] The intranuclear variety has been designated by Cowdry as Type A.[14] It is characterized by an acidophilic inclusion, margination of the basophilic chromatin on the nuclear membrane, disappearance of the nucleoli, and ultimate death, with distintegration of the involved cell.

Customarily, the viruses of this group of diseases are ectodermotropic. However, they are not restricted to skin and are apparently capable of attacking any fundamental tissue or any organ. The cutaneous lesions induced are so similar that their differentiation is almost impossible. The visceral lesions are not distinctive. The

13. Lever, W. F.: Histopathology of the Skin, Philadelphia, J. B. Lippincott Company, 1949, pp. 202-203.

14. Cowdry, E. V.: The Problem of Intranuclear Inclusions in Virus Diseases, Arch. Path. 18:527-542, 1934.

cellular reaction evoked is characterized by the aforementioned affinity of the virus for the cell nuclei, with the production of inclusions, mild inflammation with mononuclear cells predominating, and foci of granular necrosis. These changes represent the acute response of tissue to a variety of viruses rather than to a specific type. The chronic histopathologic features of visceral lesions induced by these viruses have yet to be observed and described.

Clinically, the cutaneous pattern for each disease is well established, yet at times it is difficult to distinguish one from the other. An eruption in which the vesicles arise from an erythematous base is not the sine qua non of varicella, for similar generalized eruptions occur in the herpetiform diseases. It is also well known that nonspecific systemic manifestations may accompany either disease.

The specific identification of the virus species is dependent upon laboratory studies. Although expensive and impractical for the average laboratory, animal inoculation and electron microscopy are used. Herpes simplex virus can be transmitted to the chorioallantoic membrane of the chick embryo,[15] whereas that of varicella and herpes zoster cannot be transmitted. By electron microscopy the elementary bodies of herpes simplex have a distinguishing configuration.[16] Serologically, the only test of practical diagnostic use is the complement fixation test for herpes simplex.[17] The neutralization test is too technical, costly, and time consuming. Observations are not in complete agreement on the use of the agglutination and complement fixation techniques for herpes zoster and varicella.[18]

There is fairly strong evidence for postulating a close relationship between herpes zoster and varicella. It is known that in children exposed to herpes zoster clinical varicella may develop,[19] whereas, in contradistinction, adults coming in contact with varicella are relatively immune but clinical herpes zoster may develop.[20] Furthermore, the production of clinical varicella in a healthy child by the intradermal injection with the fluid from a herpes zoster vesicle has been reported.[21]

On the basis of epidemiologic and experimental work, it is probably reasonable to conclude that the viruses represent closely related strains and that the different syndromes depend upon the properties of the strain and the tissue response of the human host.

It is obvious that etiologically, clinically, or morphologically the differentiation of disseminated herpetiform disease from varicella is not a simple matter and

15. Dawson, J. R.: Herpetic Infection of the Chorio-Allantoic Membrane of the Chick Embryo, Am. J. Path. **9**:1-6, 1933.

16. Coriell, L. L., and others: Electron Microscopy of Herpes Simplex, J. Bact. **59**:61-68, 1950.

17. Gajdusek, D. C.; Robbins, M. L., and Robbins, F. C.: Diagnosis of Herpes Simplex Infections by the Complement Fixation Test, J. A. M. A. **149**:235-240, 1952.

18. van Rooyen, C. E., and Rhodes, A. J.: Virus Diseases of Man, New York, Thos. Nelson & Sons, 1948, pp. 162-164.

19. Epidemics in Schools: An Analysis of Data Collected During the First 5 Years of a Statistical Inquiry, by the School Epidemics Committee, Medical Research Council, Special Report Series, No. 227, Her Majesty's Stationery Office, 1938, pp. 181-184.

20. Gunn, W.: Chicken-Pox, British Encyclopaedia of Medical Practice, Ed. 2, London, Butterworth & Co., Ltd., 1950, Vol. 3, p. 386.

21. Brunsgaard, E.: The Mutual Relation Between Zoster and Varicella, Brit. J. Dermat. **44**:1-24, 1932.

remains unsettled. Therefore, those cases designated as varicella in which viral studies were not done may well represent generalized herpetiform viral diseases and vice versa. It seems unusual that not one case of fatal herpes zoster with visceral lesions has been described in the literature despite its relative frequency in cases of malignancy.

CONCLUSIONS

The clinical coexistence of neoplasia and herpetiform (viral) cutaneous disease is reemphasized.

The first report of anatomic studies of a viscerally disseminated "virus" inclusional disease accompanying a neoplasm is presented.

Because of similarity, the literature of visceral involvement by the viruses of herpes simplex and varicella is reviewed and compared to the reported case.

The morphologic similarity to the lesions induced and inclusions produced by the viruses of herpes simplex, herpes zoster, and varicella emphasize the need for virus isolation studies and highly specialized serologic procedures to aid in their biologic differentiation.

ADDENDUM

Since the completion of this report, Dr. D. B. Hackel, in a paper entitled "Myocarditis in Association with Varicella," reviewed seven fatal cases (six in children, one in an adult) of clinical varicella and described the incidental occurrence of microscopic interstitial myocarditis in each. No inclusion bodies were found in these lesions, in the associated interstitial pneumonitis (three cases), or in the foci of necrosis encountered in the spleen (one case) and liver (one case). No viral isolation studies were made. In the same journal, W. J. Cheatham presented the case of a 50-year-old white woman with an established diagnosis of Hodgkin's disease who died 18 days after the onset of the pain and 7 days after the appearance of a characteristic lumbar and abdominal herpes zoster cutaneous eruption. Death was preceded by convulsions, melena, icterus, and elevation of nonprotein nitrogen of the blood. Intranuclear inclusion bodies were demonstrated within the ductal and glandular epithelium of the cardiac glands of the lower esophagus, the myenteric plexus of the stomach, dorsal root ganglia, sympathetic ganglia, the acinar lining cells of the pancreas, the cortical and medullary cells of the adrenal glands, and the stromal cells of one ovary. Foci of necrosis were described in relation to these involved sites. In addition, foci of necrosis were described within the myocardium; lungs; esophageal, gastric, and duodenal mucosae (superficial ulcers); liver lobules, and the Malpighian bodies of the spleen. However, no inclusions were described in such sites. Histoplasma capsulatum was described as an incidental finding, intracytoplasmically, within the cells of the adrenal cortex. Viral isolation studies were unsuccessful. The author emphasized the probable close relationship or identity of the viruses of herpes zoster and varicella and proposed that the same virus when spread hematogenously in a nonimmune person produces varicella, while typical zoster results from a neurogenous spread in an individual with humoral immunity.

22. Hackel, D. B.: Myocarditis in Association with Varicella, Am. J. Path. **19**:369-380, 1953.

23. Cheatham, W. J.: The Relation of Heretofore Unreported Lesions to Pathogenesis of Herpes Zoster, Am. J. Path. **19**:401-412, 1953.

GENERALIZED SALIVARY GLAND VIRUS INFECTION

Report of a Case of Its Occurrence in an Adult

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DISSEMINATION of the submaxillary gland virus in adults is unusual. Five examples have been reported to date [1]; nine others are in preparation or have been submitted for publication.[2] The clinical and pathologic findings in another instance will be described.

REPORT OF CASE

A 64-year-old white man, custodian by trade, was apparently in good health until one month before hospitalization, at which time he experienced an episode of "flu." This was characterized by fever and cough. After several days without therapy he apparently recovered. However, there were recurrences of similar episodes of cough and fever, and temperature as high as 103 F. Since the onset of this illness, there had been a weight loss of 30 to 35 lb. (13.6 to 15.9 kg.). Persistant pain, exaggerated with inspiration and near the left sternal border, appeared 24 hours before admission to the hospital. Upon admission, March 31, 1951, his cough was productive of thick, tenacious sputum. There had never been any hemoptysis.

On admission to the hospital, the patient was in a poor state of nutrition and showed evidence of much recent weight loss. The pulse rate was 80 per minute; the blood pressure was 80 systolic, diastolic unobtainable; the respirations were 20 per minute. The patient appeared to be extremely ill, and there was slight cyanosis of his lips.

Therapy consisted of oxygen by tent, aureomycin followed by chloramphenicol (Chloromycetin), and a high calory, high protein, high vitamin liquid diet.

Hospital Course.—The temperature was remittent with a diurnal variation ranging from 97 to 103 F. The pulse rate varied between 100 and 125 per minute and roughly paralleled the temperature curve. The respiratory rate never exceeded 30 per minute. Cough productive of mucoid or slightly blood-tinged sputum and persistent chest pain were the primary complaints. Sputum became rust-colored on the fifth hospital day and remained so until death. The patient became steadily weaker and lethargic and died on the 18th hospital day. Total duration of the present illness was about 48 days.

Laboratory Reports.—An x-ray (Fig. 1*A*) was interpreted by Warren Goux, M. D., on March 31, 1951 as follows: "The periphery of the right leaf of the diaphragm is elevated, but the costophrenic sulcus is not obliterated. The heart and mediastinum are displaced to the right. Adjacent to the hilum on the right is a very dense area of infiltration. This measures

1. (*a*) Langston, J. D., and Zundell, J. L.: Cytomegalic Inclusion Disease in an Adult, read before the Annual Meeting of the California Medical Association, Section on Pathology and Bacteriology, April 28, 1952. (*b*) McMillan, G. C.: Fatal Inclusion-Disease Pneumonitis in an Adult, Am. J. Path. **23**:995, 1947. (*c*) Reinhard, E. H.; Good, J. T., and Martin, E.: Chemotherapy of Malignant Neoplastic Diseases, J. A. M. A. **142**:383, 1950. (*d*) Wachstein, M.: Studies on Inclusion Bodies: Acid-Fastness of Nuclear Inclusion Bodies that Are Induced by Ingestion of Lead and Bismuth, Am. J. Clin. Path. **19**:608, 1949. (*e*) Wyatt, J. P.; Hemsath, F. A., and Soash, M. D.: Disseminated Cytomegalic Inclusion Disease in Adult with Primary Refractory Anemia and Transfusional Siderosis, Am. J. Clin. Path. **21**:50, 1951.

2. Kaplan, L.: Personal communication to the author. Steiner, R. C., and Reingold, I. M.: Personal communication to the author. Wyatt, J. P., and Trumbull, M. L.: Cytomegalic Inclusion Pneumonitis in the Adult, read before the Thirty-first Annual Meeting of the American Society of Clinical Pathologists, Chicago, Oct. 16, 1952.

5 cm. in diameter. Radiating from this area are many coarse bronchovascular markings. A 5 cm. area of radiolucency partially overlaps both this dense area and the cardiac silhouette. An area of increased radiolucency is also present in the axilla adjacent to the fifth rib. On the left, the hilum is enlarged, and a soft, mottled infiltration extends laterally and toward the base."

Another chest film, four days later (Fig. 1*B*), showed considerable change in the appearance of both lung fields: "The intensely consolidated area on the right lung is much less dense, but the involvement is more widespread. There is no longer any evidence of pneumothorax. The heart remains displaced to the right. On the left, infiltration is more dense around the hilum and also in the periphery of the base."

A cytologic examination of sputum failed to reveal tumor cells.

The electrocardiogram was not specific and was interpreted as showing a definitely abnormal curve with evidence of myocardial damage.

Blood studies on March 31 gave the following values: hematocrit reading 47% per 100 ml.; hemoglobin 14.2 gm. per 100 ml.; white blood cells 8,450, with 10% stab forms, 66% segmented

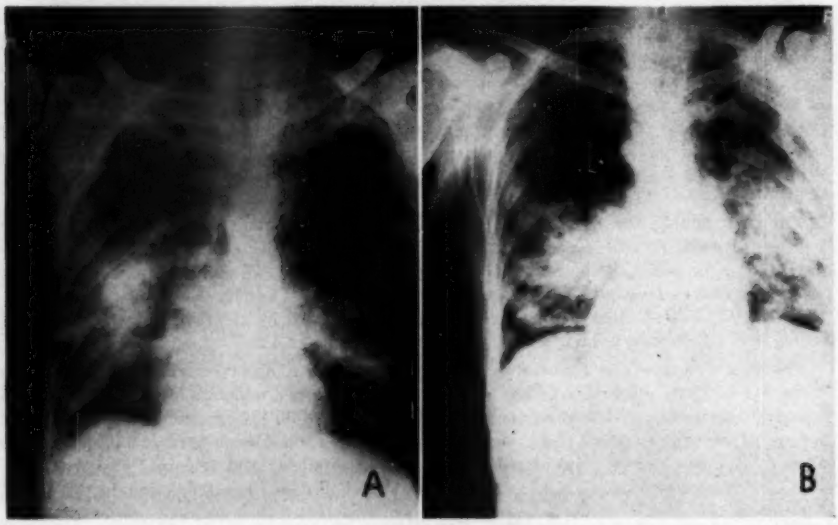

Fig. 1.—*A*, roentgenogram of lungs on day after admission to hospital. *B*, roentgenogram showing pulmonary changes four days following admission to the hospital.

forms, 19% lymphocytes, 3% monocytes, 1% basophiles, and 1% eosinophiles. The sedimentation rate was 4 mm. per hour (Wintrobe).

Urinalysis, March 31, showed 1+ albumin; 5 to 10 white blood corpuscles per high-power field, and occasional granular and hyaline casts.

The coccidioidin skin test was negative. Serologic reactions for syphilis were negative.

No acid-fast bacilli were demonstrated in the sputum, but Candida albicans and Bacillus subtilis were cultured.

Autopsy.—The body was that of an emaciated white man, appearing about the stated age of 64. There was slight nonpitting edema of the ankles.

When the sternum was reflected an abscess cavity 12 cm. in diameter was opened. It was located in the medial anterior inferior portion of the upper lobe of the left lung, had a distinct gray-white capsule with a finely granular gray lining, and was filled with thick gray nonodorous material. It was closely adherent to the inferior aspect of the sternum.

Within the lower lobe of the left lung were several nodules, from 0.5 to 1 cm. in diameter, which were well circumscribed, granular, and had a yellowish tinge. One was subpleural, and the others were within the substance of the lung. In addition, within the upper portion of the lower lobe of the left lung and within the upper and middle lobes of the right lung were areas up to 5 cm. in diameter which were firm and on section appeared alternately gray-red and yellow. They were predominantly subpleural and grossly resembled infarcts. Adjacent to one in the apical portion of the upper lobe of the right lung was a small pulmonary artery containing an adherent thrombus. There was a moderate degree of fusiform bronchiectasis of the lower lobe of the right lung.

There was brown atrophy of the heart and an old myocardial infarction of the anterior two-thirds of the interventricular septum. The coronary ostia were concentrically narrowed by atheromatous plaques.

The spleen was approximately four times normal size and had a smooth blue capsule and a firm dark red-blue pulp.

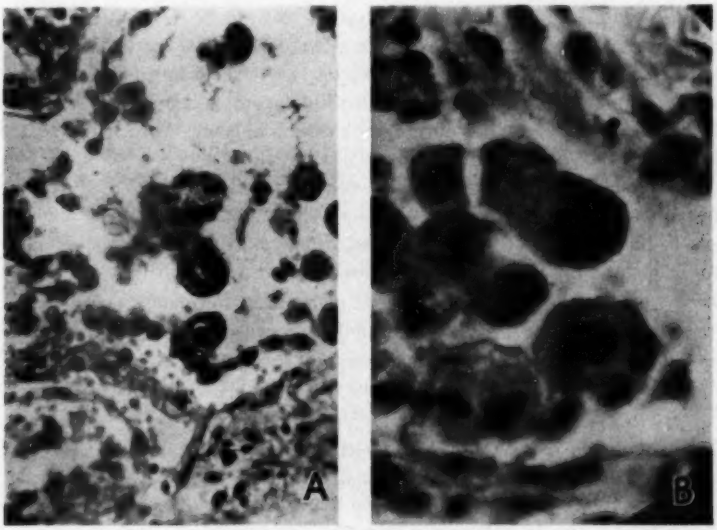

Fig. 2.—*A*, hypertrophy of alveolar lining cells and large intra-alveolar cells with intranuclear inclusions. Hematoxylin and eosin; × 250. *B*, large cells with intranuclear inclusions and intracytoplasmic inclusions. Prominent intracytoplasmic inclusions in cell in lower right-hand portion of illustration. Hematoxylin and eosin; × 970.

There were no significant abnormalities of the liver, kidneys, pancreas, or gastrointestinal tract.

Both adrenals were very firm and red. On section, both cortices were alternately bright yellow and hemorrhagic.

MICROSCOPIC EXAMINATION

Throughout the lung were numerous areas of necrosis characterized by complete loss of lung architecture and only ghosts of inflammatory cells and occasional lymphocytes. The surrounding alveoli had thickened walls lined by columnar to cuboidal cells. In some portions there appeared to be budding-off of giant alveolar lining cells. Areas were present where large numbers of these cells were free within alveolar spaces. Occasional large cells had two nuclei. Within many of the nuclei of these large cells were round to oval inclusion bodies, the smaller tending

to be round, whereas larger ones were oval or slightly indented. Larger inclusions appeared to completely fill the nuclei except for a thin zone of clearing between the edges of the incisions and the nuclear membranes. This gave these cells an "owl-eye" appearance. With hematoxylin and eosin stains, the intranuclear inclusions were basophilic, although some had a slightly eosinophilic tinge.

Within the cytoplasm of many of the cells with intranuclear inclusions were eosinophilic to faintly basophilic smaller inclusions. These were 2 to 4 μ in diameter, and some were surrounded by clear halos.

Many areas which grossly resembled pulmonary infarcts consisted of similar areas of necrosis, organizing pneumonia, and giant cells with inclusion bodies. In areas of early necrosis very few, if any, cells with inclusion bodies were noted. Instead, there were occasional red blood corpuscles, polymorphonuclear leucocytes, and great numbers of round cells. Inclusions were in greatest numbers in areas resembling organizing pneumonia and adjacent to zones of necrosis. A single intranuclear inclusion body was noted within a ciliated bronchial epithelial lining cell.

A Hotchkiss-McManus stain for mucopolysaccharides failed to stain the intranuclear inclusion bodies. However, the small intracytoplasmic bodies stained deeply.

The intranuclear as well as intracytoplasmic inclusions were negative for combined or free iron. None of the inclusions were acid-fast.

Microscopically, there was extensive necrosis of the adrenal medulla, the zona reticularis, and inner portions of the zona fasciculata. Scattered cortical cells adjacent to the zones of necrosis contained similar but, on the whole, smaller intranuclear inclusions than those noted within the lungs.

There were no significant microscopic changes in the pancreas.

Occasional granular, slightly pigmented casts were found within renal tubules.

A moderate amount of myeloid metaplasia of the spleen was present.

COMMENT

There have been many reports of disseminated cytomegalic inclusion disease in infants with involvement of various organs,[3] with suggestions by some authors that the infection may occur "in utero."[3d,e] In many instances the dissemination apparently was related to or followed various virus infections, such as measles and whooping cough.[4] Smith and Vellios [3d] concluded that in children under 2 months of age generalized salivary gland virus infection is usually the primary fatal disease, with frequent clinical and anatomic manifestations of blood dyscrasia or hepatic damage. Frequently present in the 20 cases of generalized cytomegalic inclusion disease reported by Smith and Vellios was interstitial or chronic pneumonia. Farber and Wolbach [3a] found that 22, or 12%, of the submaxillary glands in 183 infants contained the submaxillary gland virus. Because of the relatively common finding

3. (*a*) Farber, S., and Wolbach, S. B.: Intranuclear and Cytoplasmic Inclusions ("Protozoan-Like Bodies") in the Salivary Glands and Other Organs of Infants, Am. J. Path. **8**:123, 1932. (*b*) Goodpasture, E. W.; Auerbach, S. H.; Swanson, H. S., and Cotter, E. F.: Virus Pneumonia of Infants Secondary to Epidemic Infections, Am. J. Dis. Child. **57**:997, 1939. (*c*) Kinney, T. D.: Intranuclear Inclusions in Infancy, Am. J. Path. **18**:799, 1942. (*d*) Smith, M. G., and Vellios, F.: Inclusion Disease of Generalized Salivary Gland Virus Infection, Arch. Path. **50**:862, 1950. (*e*) Wyatt, J. P.; Saxton, J.; Lee, R. S., and Pinkerton, H.: Generalized Cytomegalic Inclusion Disease, J. Pediat. **36**:271, 1950.

4. Footnote *3b*, *c*, *d*, and *e*.

of this virus in submaxillary glands of newborn infants and because of the frequent association of other diseases, it was suggested that generalized salivary gland virus infection may often be a terminal phenomenon with dissemination of the virus as a result of debilitation from some other primary disease.[3d, e]

Five examples of disseminated cytomegalic inclusion disease in adults have been described to date.[1] In von Glahn's case,[1d] the first to be reported in adults, the disease was associated with various inflammatory foci. In the lungs the giant inclusion bodies were most profuse near areas of pneumonia. Within the liver they were adjacent to a large liver abscess. Similar inclusions were noted in the region of an ulcer of the colon. McMillan's patient,[1b] a 60-year-old woman who presented clinical symptoms similar to interstitial pneumonia, was also believed to have a vitamin A deficiency. Two other adults with generalized cytomegalic inclusion disease [1c, e] suffered from hematologic disorders, one with primary refractory anemia,[1e] and the other with leucosarcoma of the subleukemic variety.[1e]

The patient here presented died with generalized salivary gland virus infection involving the lungs and adrenals. The inclusions were in greatest profusion and were intimately associated with areas of organizing pneumonia and pulmonary necrosis. The extensive bilateral adrenal necrosis was the immediate cause of death. Adrenal necrosis was also described in the case reported by Wyatt and associates.[1e] The 60-year-old woman reported by McMillan [1b] presented x-ray findings of a progressive peribronchial pneumonitis of an unusual nature, very similar to that in our patient.

The myeloid metaplasia of the spleen and the pigmented renal casts suggest a hemolytic process.

SUMMARY

An example of generalized salivary gland virus infection in a man involving the lungs and suprarenals with bilateral suprarenal necrosis is reported.

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News and Comment

American Cancer Society.—The American Cancer Society has announced that three additional institutions have been approved by its Board of Directors for training American Cancer Society Fellows in Exfoliative Cytology. These institutions and the Directors of the Laboratory of Exfoliative Cytology are as follows: Memorial Cancer Center, New York, Howard Richardson and Emerson Ray; The Roosevelt Hospital, New York, Walter W. Brandes; The Jewish Hospital, St. Louis, Herman T. Blumenthal.

Further information concerning the Society's support in training qualified pathologists in this field may be procured from Brewster S. Miller, M.D., Director, Professional Education Section, American Cancer Society, 47 Beaver St., New York 4.

Books

Taschenbuch der praktischen Medizin. Volume 8. Edited by Dr. J. Kottmaier, Baden-Baden, Germany. Second edition. Price, 29.70 German marks. Pp. 937. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O.

Probleme der Morphologie, Cytochemie und Wuchsform des Tuberkuloseerregers. By Dr. F. J. Bassermann, Honnef/Rhine, Germany. Price, 7.80 German marks. Pp. 98, with 40 illustraions. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O.

Aviation Toxicology: An Introduction to the Subject and a Handbook of Data. Prepared under the direction of the Committee on Aviation Toxicology, Aero Medical Association. Price, $4.00. Pp. 120, with 17 tables and 7 illustrations. The Blakiston Company, 1012 Walnut St., Philadelphia 5, 1953.

Progress in Biophysics and Biophysical Chemistry. Volume 3. Edited by J. A. V. Butler, Professor of Physical Chemistry, University of London; Chester Beatty Research Institute, Royal Cancer Hospital, London, and J. T. Randall, F.R.S., Wheatstone Professor of Physics at the University of London at King's College. Price, $9.50. Pp. 386. Academic Press, Inc., 125 E. 23rd St., New York; Pergamon Press, Ltd., London, 1953.

Die Zusammenarbeit des Geburtshelfers, des Kinderarztes und des Serologen bei der Prophylaxe und Therapie der Neugeborenen-Erythroblastose. By Prof. Dr. P. Dahr, Göttingen; Prof. Dr. K. H. Schäfer, Hamburg; Priv.-Doz. Dr. A. Stadtmüller, Göttingen. Price 5.70 German marks. Pp. 64, with 10 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1953.

Overeating, Overweight and Obesity. Proceedings of the Nutritional Symposium Held at the Harvard School of Public Health, Boston, Massachusetts, October 29, 1952: Nutrition Symposium Series Number 6. By David P. Barr, John R. Brobeck, Henry W. Brosin, Louis I. Dublin, Frank A. Evans, P. C. Fry, Samuel Gurin, Paul György, Edward E. Hunt Jr., Ancel Keys, P. S. Peckos, and A. W. Pennington. Price, $1.50. Pp. 151. The National Vitamin Foundation, Inc., 150 Broadway, New York 38, 1953.

Surgical Forum: Proceedings of the Forum Sessions, Thirty-Eighth Clinical Congress of the American College of Surgeons, New York City, September, 1952. Surgical Forum Committee: Owen H. Wangensteen, M.D., F.A.C.S., Minneapolis, Chairman; J. Garrott Allen, M.D., F.A.C.S., Chicago; Warren H. Cole, M.D., F.A.C.S., Chicago; Robert E. Gross, M.D., F.A.C.S., Boston; Carl A. Moyer, M.D., F.A.C.S., Dallas; I. S. Ravdin, M.D., F.A.C.S., Philadelphia; H. B. Shumacker, M.D., F.A.C.S., Indianapolis. Price, $10.00. Pp. 716, illustrated. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

Effective Inhalation Therapy. By Edwin Rayner Levine, M.D., Chairman of the Committee on Physiologic Therapy, American College of Chest Physicians; Formerly Director of the Chest Service, Michael Reese Hospital, Chicago, with the cooperation of Alvan L. Barach, M.D., Clinical Professor of Medicine, College of Physicians and Surgeons, Columbia University; Associate Attending, Fresbyterian Hospital, New York; J. Winthrop Peabody, M.D., Professor of Diseases of the Respiratory System, Georgetown University Medical School, Washington, D. C., and Maurice S. Segal, M.D., Clinical Professor of Medicine, Tufts College Medical School, Boston; Director of the Department of Inhalational Therapy, Boston City Hospital. Price, $4.50. Pp. 157, with 49 illustrations in color. National Cylinder Gas Company, Medical Division, Chicago 11, 1953.

Gifford's Textbook of Ophthalmology. By Francis Heed Adler, M.D., Professor of Ophthalmology, University of Pennsylvania Medical School; Consulting Surgeon, Wills Eye Hospital, Philadelphia. Fifth edition. Price, $7.50. Pp. 488, with 281 illustrations and 26 color plates. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

The fifth edition of this popular textbook for medical students has considerably enlarged sections on medical ophthalmology, especially the thirty-two pages devoted to hypertensive diseases. The author's great interest in physiology is reflected in probably the best recent correlation of anatomy, physiology, and pathology of hypertensive vascular disease, with the retinal picture. A chapter of twenty-nine pages on the "Ocular Manifestations of General Diseases" includes many infectious diseases, the arthritides, blood dyscrasias, and an enlarged section on diabetes. The underlying neurologic basis for disorders of motility is stressed. The text is written in the author's usual clear style; the many illustrations are well chosen, and the format is good. Because of the emphasis on the basic science aspects of ocular disease, this text can be recommended for those in nonclinical fields.

Synovial Fluid Changes in Joint Disease. By Marian W. Ropes, M.D., Associate Physician, Massachusetts General Hospital; Assistant Clinical Professor of Medicine, Harvard Medical School, and Walter Bauer, M.D., Chief of Medical Services, Massachusetts General Hospital; Jackson Professor of Clinical Medicine, and Director of Robert W. Lovett Memorial Foundation for the Study of Crippling Disease, Harvard Medical School. Price, $4.00. Pp. 150, with 15 figures and 14 tables. Published for the Commonwealth Fund by Harvard University Press, Cambridge 38, Mass., 1953.

The detailed and painstaking studies on synovial fluid that have been undertaken for the past 20 years at the Massachusetts General Hospital have been summarized and analyzed very effectively in the present volume, which should represent for some time the last, or almost the last, word on the subject. It was the original thesis that disease in the synovia would be reflected by the composition of the synovial fluid; the findings have borne this out to the extent of differentiating between traumatic arthritis and nontraumatic joint disease. These studies have coincided with a broadening of knowledge, to which the authors have indeed contributed largely, of the nature of mucinous substances and of the enzymes that affect them. They cover also the period in which management of the arthritic diseases has been revolutionized by treatment with the corticoid hormones. Remissions so induced cause the synovial fluid to revert to a more normal composition. Only briefly touched on in this account are the microscopic changes in the synovialis. A correlation of histochemical events in the lesion with chemical and physicochemical changes in the exudate appears to be the next step in the analysis pioneered by Ropes and Bauer.

Regeneration and Wound-Healing. By A. E. Needham, M.A., B.Sc., University Demonstrator in Zoology, University of Oxford. Price, $1.75. Pp. 152, with 5 illustrations and 3 tables. John Wiley & Sons, Inc., 440 4th Ave., New York 16, 1952.

The orientation of this compact and informative monograph is rather strongly zoological, and a glossary of several of the terms used would have been a convenience for the general reader. Its scope ranges from repair of wounds in the higher animals, through the regeneration of whole limbs in Crustacea, to the development of entire organisms from minute fragments (for example, a hydra from a $\frac{1}{200}$th fragment). There are no tidy answers to these problems. The author displays a rather extreme "biochemicist" point of view, and one picks up such sentences as the following: "The complete understanding of regeneration demands ultimately a knowledge of the rôle of every relevant metabolite, including inorganic substances, and until it is proved otherwise every substance shown to affect regeneration must be considered relevant either in itself or as a proxy." Apart from the implied tautology, such a view would effectively paralyze investigation. It ignores processes of homeostasis and the concept of the first approximation. It may also be noted that much work is going forward on mesenchymal structure and its reactions to injury and stimulation that does not find a place in this account.

Textbook of Histology. By Jose F. Nonidez, D.Sc., Late Professor of Anatomy, Cornell University, and Professor of Microscopic Anatomy, University of Georgia, and William F. Windle, Ph.D., Sc.D., Scientific Director, Baxter Laboratories, Inc., Formerly Professor of Anatomy, University of Pennsylvania, and Professor of Microscopic Anatomy and Professor of Neurology, Northwestern University. Second edition. Price, $9.50. Pp. 528, with 326 illustrations composed of 214 drawings and diagrams and 233 photomicrographs. McGraw-Hill Book Company, Inc., 330 W. 42nd St., New York 18, 1953.

The general plan in this edition follows that of the first edition, but many improvements have been incorporated. New descriptive material, new tables, and new bibliographic references have been added. The excellent photomicrographs of the first edition have been retained and have been supplemented by several new ones.

This appears to be an excellent, although an expensive, text for use in undergraduate courses in human histology. It is brief but concise and is well illustrated. The lack of color in the illustrations is regrettable, especially in the chapters on blood and blood formation. The authors suggest that this text is suitable for use in courses of medical histology, but it seems its use in a course at this level would require skillful use of extensive supplementary material.

Atlas of Tumor Pathology: Section IV, Fascicle 14, Tumors of the Thyroid Gland. By Shields Warren, M.D., Professor of Pathology, Harvard Medical School; Pathologist, New England Deaconess Hospital, and William A. Meissner, M.D., Assistant Professor of Pathology, Harvard Medical School; Associate Pathologist, New England Deaconess Hospital, Boston. Price, $1.75. Pp. 97, with 82 illustrations and 5 tables. Published by the Armed Forces Institute of Pathology, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, 2101 Constitution Ave., N.W., Washington 25, D. C., 1953.

This very excellent analysis of tumors of the thyroid gland is a well-balanced presentation that merits the highest praise. It provides an acceptable standard nomenclature, clear and instructive illustrations, a concise authoritative text, and an adequate bibliography. There is a preliminary brief discussion of "tumor-like lesions." The difficulty here lies in providing adequate differentiation between a hyperplastic condition with "tumor-like" nodules and the true adenoma. This portion of the text is perhaps overschematized, so that the elementary student might not realize the complexity of the problem. The authors adhere throughout to a simple nomenclature that should gain general acceptance. In the main portion of the text the types of adenoma and of carcinoma and the specific problems that arise therefrom are lucidly discussed. The separate conditions are treated as biological problems, each with its own natural history. The text, although brief, is highly informative. A few of the illustrations are of inferior grade but in general are excellent. Altogether the volume deserves a widespread circulation.

Morris' Human Anatomy: A Complete Systematic Treatise. Edited by J. Parsons Schaeffer, A.M., M.D., Ph.D., Sc.D., D.Litt., Formerly Professor of Anatomy and Director of The Daniel Baugh Institute of Anatomy, Jefferson Medical College, with contributions from 15 other authors. Eleventh edition. Price, $16. Pp. 1,718, with 1,220 illustrations. The Blakiston Company, 1012 Walnut St., Philadelphia 5; 105 Bond St., Toronto 2, Canada, 1953.

The eleventh edition of this well-known textbook appears after an interval of eleven years since the last revision. The general characteristics have been largely retained, although several sections were prepared by new collaborators. There is a welcome addition of roentgenograms and of other new illustrations. The quality of some halftones and of color reproductions is not always first class. Functional considerations, descriptions of variations, and the discussions of clinical aspects, in general, are set in double columns. Certain chapters appear to have been more thoroughly revised than others. This text is of a rather encyclopedic nature and constitutes an excellent reference book. It makes no concessions to shortened curricula or learning time. This, perhaps, is best illustrated by the length and completeness of certain sections, for example, Osteology and Articulations (309 pages), the Nervous System (312 pages). The book is larger by eighty-three pages than the previous edition. Fairly extensive references for collateral reading are appended to each section. The index has been much improved and simplified.

The Physician in Atomic Defense: Atomic Principles, Biologic Reaction and Organization for Medical Defense. By Thad P. Sears, M.D., F.A.C.P., Associate Clinical Professor of Medicine, University of Colorado School of Medicine; Chief of Medical Service, Veterans Administration Hospital, Denver; Member of Advisory Staff to Director of Civil Defense, State of Colorado; Member of Disaster Commission, Colorado State Medical Society; Colonel, Medical Corps, United States Army Reserve. Price, $6. Pp. 308, with 53 illustrations. Year Book Publishers, Inc., 200 E. Illinois Street, Chicago 11, 1953.

In this day and age, when it has become difficult enough to keep abreast of the literature in one's own field of specialty and, unhappily, of medicine in general, it is manifestly impossible for most of us to give much time and thought to serious study of hybrids such as the borderlands of physics and medicine. When, therefore, a volume such as this appears, it should be greeted with satisfaction and a measure of relief, for it brings together in one place and in readable form what could otherwise be found only through diligent search and critical review of a vast amount of literature. Dr. Sears has accomplished an ambitious assignment that was borne of his conviction that a certain amount of basic knowledge in this borderline territory is necessary for most of us and especially for the physcian who may be called upon to treat injuries and illness resulting from an atomic catastrophy. To this end the author has systematically ordered and carefully described what to him has seemed pertinent for his own understanding of this complex field. He has accomplished his objective as stated in the introduction, to wit, "The objective of this volume is, first, to review briefly the present physical concepts of matter, gaining in this approach and without recourse to mathematical formulas, a sounder understanding of radioactivity and its applications. Attention will next be given the structure of the atom; the significance of radioactivity; the chain reaction and the atomic bomb; the use of isotopes. Then the symptomatology, pathology and treatment of atomic bomb injuries and the methods of civil defense will be reviewed."

The book will serve a dual purpose to advantage. It will provide interesting and enlightening reading to the person who has a desire to become acquainted with the physical background of what one might call atomic medicine in its broadest sense. It will also serve as a useful and reliable source book to be consulted when the occasion arises on matters ranging from transformation of nuclear particles to mortuary functions in atomic defense. The book is clearly written, though of necessity sketchy at times. Many diagrams, figures, and photographs have been added to convey a visual image of seemingly remote and often mysterious-appearing cosmic and not so cosmic processes as they relate to the realities of life in the twentieth century. There are many references at the end of each chapter.

The Surgery of Infancy and Childhood: Its Principles and Techniques. By Robert E. Gross, M.D., D.Sc., William E. Ladd Professor of Children's Surgery, Harvard Medical School; Chief of Surgical Service, Children's Hospital, Boston. Price, $16.00. Pp. 1,000 with 1,488 illustrations. Drawings by Etta Piotti. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

This volume respresents thorough coverage for all the important conditions in pediatric surgery, with the exception of trauma. Special attention is given to congenital anomalies, which are so common and so often require urgent and specific surgical therapy. None of the less common conditions, such as cardioesophageal chalasia, have been omitted. Very appropriately, a separate chapter is devoted to premature infants; still more appropriate is a long chapter of thirty-one pages on preoperative and postoperative care. Dr. Gross makes the very emphatic statement that the preoperative and postoperative care should be the responsibility of the surgical service as it has been at Children's Hospital, Boston, for many years.

The amount of space devoted to each subject is very suitable. Naturally, congenital lesions of the heart and great vessels are adequately covered, since the author has a particular interest in this phase of pediatric surgery and has made many valuable contributions in this field. However, one cannot say that too much space has been devoted to this very important specialty; if any subject has been given more space, comparatively, than others, it is probably urology.

The text is extremely well written and reads readily. The author shows unusual familiarity with modern thoughts in pediatric surgery, thus proving that he has had vast experience in his field. Operative technique is well covered and amply illustrated with excellent illustrations. Manifestations, complications, et cetera are adequately discussed. Of particular value after discussion of all important subjects are the statistical data assembled from his hospital records; these data give the reader a splendid conception of what results should be obtained, what complication should be expected, et cetera. An excellent modern bibliography is included at the end of each chapter.

In summary, this volume is a near classic in its field. It will be very popular with experienced surgeons, as well as students and residents in training. It is a fine contribution to surgery, and the author is to be congratulated.

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Purpose

Laboratory administrators interested in the health of their laboratory personnel have recently become concerned with infectious aerosols sometimes encountered during centrifuging. Infectious materials sealed in centrifuge tubes or bottles quickly become airborne when they are released by a leak or fracture of the container due to high centrifugal forces. The natural draft created by the fan action of the rotating head acts to distribute the material through the laboratory.

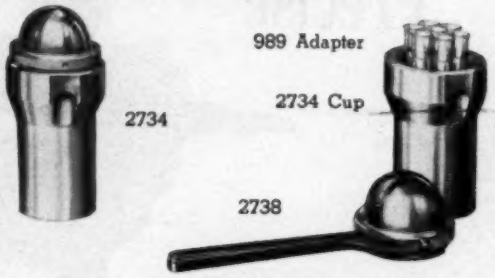
Approach

International Equipment Company engineers have adapted standard accessories to permit sealing of the centrifuge cup. To insure maximum safety, the scientist having completed centrifuging removes the sealed container to a hood for opening. In those instances where a leak or fracture has allowed the material to contaminate the container, immediate decontamination steps may be taken.

Solution

A series of accessories based upon the Hi-speed 250 ml. slotted cup has been designed. The standard cup is threaded to take a dome cover fitted with an O-ring seal. The cup holds one of the standard round or flat bottom 250 ml. centrifuge bottles. Adapters are available for holding: 2–50 ml. centrifuge tubes; 7–15 ml. centrifuge tubes; and 10–13 x 100 mm. serum tubes.

Centrifuge	Head	No. Cups	R.P.M.	R.C.F.
Size 1, Model SBV	277	4	3000	2000
Size 2, Model V...	277	4	2900	1880
Size 2, Model V...	266	6	2300	1410
Model PR-1.....	284	4	2400	1240



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2738 — Wrench, spanner type for tightening screw dome on 2734 — $1.75.

2741 — Adapter, 2-place, for 50 ml. centrifuge tubes, fits 2734 or any standard IEC 250 ml. centrifuge cup — $4.50.

989 — Adapter, 7-place, for 15 ml. centrifuge tubes, fits 2734 or any standard IEC 250 ml. centrifuge cup — $18.00.

2740 — Adapter, 10-place, for 13 x 100 mm. serum tubes, will fit 2734 or any standard IEC 250 ml. centrifuge cup — $21.50.

277 — Head, 4-place, steel pin type for 2734 or 384 cups — $63.00.

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